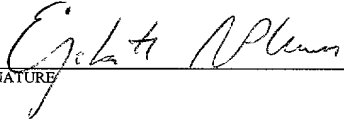


FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV. 1094) TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER B0192/7019 09/581500 <small>U.S. APPLICATION NO. (If known, see 37 CFR 1.5)</small>
INTERNATIONAL APPLICATION NO. PCT/EP98/08543	INTERNATIONAL FILING DATE 17 December 1998 (17.12.98)	PRIORITY DATE CLAIMED 18 December 1997 (18.12.97)
TITLE OF INVENTION MOOD DISORDER GENE		
APPLICANT(S) FOR DO/EO/US VAN BROECKHOVEN, Christine, RAEYMAEKERS, Peter, DEL-FAVERO, Jorgen		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This express request to begin national procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)) with verification of translation. 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(C)(5)). 		
Items 11. To 16. Below concern document(s) or information included:		
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98 with references. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 14. <input type="checkbox"/> A substitute specification (submitted as a first Preliminary Amendment). 15. <input type="checkbox"/> A change of power of attorney and/or address letter. 16. <input checked="" type="checkbox"/> Other items or information: Copy of International Preliminary Examination Report Copy of International Published Application Copy of International Search Report Copy of PCT Request form PCT/RO/301 Copy of Sequence Listing (paper copy) Express Mail Label No. EL437904005US Mailed June 14, 2000 		

U.S. APPLICATION NO. (if known, see 37 CFR 1.53)		INTERNATIONAL APPLICATION PCT/EP98/08543		ATTORNEY'S DOCKET NUMBER B0192/7019	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):					
Search Report has been prepared by the EPO or JPO \$840.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).. \$690.00					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$970.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$840.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 X 30 months from the earliest claimed priority date (37 CFR 1.492(e)).					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	50- 20 =	30	X \$18.00	\$540.00	
Independent Claims	6-3 =	3	X \$78.00	\$234.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+\$260.00	\$260.00	
TOTAL OF ABOVE CALCULATIONS =				\$	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate coversheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED =				1,874.00	
				Amount to be:	
				refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1874.00</u> to cover the above fees is enclosed.					
b. Please charge by Deposit Account No. _____ In the amount of \$ _____ To cover the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/> The commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO				SIGNATURE	
Elizabeth R. Plumer WOLF, GREENFIELD & SACKS, P.C. 600 Atlantic Avenue Boston, Massachusetts 02210					
				NAME	
				Elizabeth R. Plumer	
				REGISTRATION NO	
				36,637	

ATTORNEY'S DOCKET NO. B0192/7019(ERP)
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: VAN BROECKHOVEN, Christine, et al.
Serial No: Not Yet Assigned
Filed: Herewith
International Application No.: PCT/EP98/08543
International Filing Date: 17 December 1998 (17.12.98)
Priority Date Claimed: 18 December 1997 (18.12.97)
For: MOOD DISORDER GENE

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please enter the Preliminary Amendment as follows:

In the Specification:

Please amend the specification on page 1, immediately following the title by inserting the following:

--

Related Applications

This application claims foreign priority benefits under Title 35, U.S.C., §119(a)-(d) or §365(a),(b) of PCT application no. PCT/EP98/08543, filed December 17, 1998, and foreign patent application no. GB 9726804.9, filed December 18, 1997, the entire contents of which are incorporated herein by reference.--

On page 67, line 21, please add --All references disclosed herein are incorporated by reference in their entirety.--

In the Claims:

Please amend claims 4, 6, 8, 9, 11, 13, 14, 16, 17, 19, 28, 40, 41, 43, 44, 45, and 47, before calculating the filing fee as follows:

4. (Amended) The use as claimed in claim 2 [or 3] wherein said YAC clone is 961_h_9, 942_c_3, 766_f_12, 731_c_7, 907_e_1, 752-g-8 or 717_d_3.

6. (Amended) The use as claimed in claim 1 [any preceding claim] wherein said mood disorder or related disorder is selected from the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV) taxonomy and includes mood disorders (296.XX,

300.4, 311, 301, 13, 295.70), schizophrenia and related disorders (295, 297.1, 298.9, 297.3, 298.9), anxiety disorders (300.XX, 309.81, 308.3), adjustment disorders (309, XX) and personality disorders (codes 301. XX).

8. (Amended) A method of identifying at least one human gene, including mutated or polymorphic variants thereof, which is associated with a mood disorder or related disorder which comprises fragmentation of a YAC clone as defined in claim 2 [any one of claims 2 to 4] and detection of nucleotide triplet repeats.

9. (Amended) A method as claimed in claim 7 [or 8] wherein said repeated triplet is CAG or CTG.

11. (Amended) A method of identifying at least one human gene including mutated or polymorphic variants thereof, which is associated with a mood disorder or related disorder wherein said gene is present in the DNA comprised in the YAC clones as defined in claim 2 [any one of claims 2 to 5], which method comprises the step of detecting an expression product of said gene with an antibody capable of recognising a protein with an amino acid sequence comprising a string of at least 8 continuous glutamine residues.

13. (Amended) A method as claimed in claim 11 [or claim 12] wherein said antibody is mAB 1C2.

14. (Amended) A method of preparing a contig map of YAC clones of the region of human chromosome 18q between polymorphic markers D18S60 and D18S61 which comprises the steps of:

(a) subcloning the YAC clones according to claim 2 [any one of claims 2 to 5] into exon trap vectors;

(b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other known sequence tagged sequence from the YAC contig described herein, or part thereof consisting of not less than 14 contiguous bases or the complement thereof, to detect overlaps among the cosmid vectors, and

(c) constructing a cosmid contig map of a YAC clone of said region.

16. (Amended) A method of identifying at least one human gene or mutated or polymorphic variants thereof which is associated with a mood disorder or related disorder which comprises the steps of:

(a) subcloning the YAC clones according to claim 2 [any one of claims 2 to 5] into a cosmid, BAC, PAC or other vector;

(b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other known sequence tagged sequence from the YAC contig described herein, or part thereof consisting of not less than 14 contiguous bases or the complement thereof, to detect overlaps amongst the subclones and construct a map thereof;

(c) identifying the position of genes within the subcloned DNA by one or more of CpG island identification, zoo-blotting, hybridization of said subcloned DNA to a cDNA library or a Northern blot of mRNA from a panel of culture cell lines;

(d) detecting differences between said genes and equivalent regions of the DNA of an individual afflicted with a mood disorder or related disorder; and

(e) identifying said gene which, if defective, is associated with said mood disorder or related disorder.

17. (Amended) An isolated human gene, including mutated or polymorphic variants thereof, which is associated with a mood disorder or related disorder which is obtainable by the method according to claim 7 [any of claims 7 to 13, 15 or 16].

19. (Amended) A cDNA encoding the protein of claim 18 which is obtainable by the method of claim 7 [any one of claims 7 to 13, 15 or 16].

28. (Amended) A method as in claim 26 [or 27] which comprises the steps of:

a) obtaining a DNA sample from said individual;

b) providing primers suitable for the amplification of a nucleotide sequence comprised in the sequence shown in any one of Figures 15a, 16a, 17a or 18a said primers flanking the trinucleotide repeats comprised in said sequence;

c) applying said primers to the said DNA sample and carrying out an amplification reaction;

d) carrying out the same amplification reaction on a DNA sample from a control individual; and

e) comparing the results of the amplification reaction for the said individual and for the said control individual;

wherein the presence of an amplified fragment from said individual which is bigger in size from said individual which is bigger in size from that of said control individual is an

indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

40. (Amended) An expression vector which comprises a sequence of nucleotides as claimed in claim 21 [claims 21 or 22].

41. (Amended) A reporter plasmid which comprises the promoter region of a nucleic acid molecule as claimed in claim 21 [or 22] positioned upstream of a reporter gene which encodes a reporter molecule so that expression of said reporter gene is controlled by said promoter region.

43. (Amended) An eukaryotic cell or multicellular tissue or organism comprising a transgene encoding a protein as claimed in claim 23 [claims 23 or 24].

44. (Amended) A method for determining if a compound is an enhancer or inhibitor of expression of a gene associated with a mood disorder or related disorder which comprises the steps of:

- a) contacting a cell as claimed in claim 42 with said compound;
- b) detecting and/or quantitatively evaluating the presence of any mRNA transcript corresponding to a nucleic acid as claimed in claim 21 [or 22]; and
- c) comparing the level of transcription of said nucleic acid with the level of transcription of the same nucleic acid in a cell as claimed in claim 42 not exposed to said compound[;].

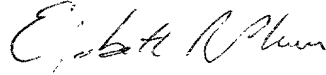
45. (Amended) A method for determining if a compound is an enhancer or inhibitor of expression of a gene associated with a mood disorder or related disorder which comprises the steps of:

- a) contacting a cell as claimed in claim 42 with said compound;
- b) detecting and/or quantitatively evaluating the expression of a protein as claimed in claim 23 [claims 23 or 24]; and
- c) comparing the level of expression of said protein with that of the same protein in a cell not exposed to said compound.

47. (Amended) A compound identified as an enhancer or an inhibitor of the expression of a gene associated with a mood disorder or related disorder by a method as claimed in claim 44 [claims 44 to 46].

If any other information is needed, please contact the undersigned attorney by phone (617-720-3500, Ext. 343) to expedite the further prosecution of this patent application.

Respectfully submitted,



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ERP/ses
Docket No. B0192/7019(ERP)
Date: June 14, 2000
X06/017/00

7/PART

09/581500

533 Rec'd PCT/PTO 14 JUN 2000

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MOOD DISORDER GENE

The invention is concerned with the determination of genetic factors associated with psychiatric health with particular reference to a human gene or genes which contributes to or is responsible for the manifestation of a mood disorder or a related disorder in affected individuals. In particular, although not exclusively, the invention provides a method of identifying and characterising such a gene or genes from human chromosome 18, as well as genes so identified and their expression products. The invention is also concerned with methods of determining the genetic susceptibility of an individual to a mood disorder or related disorder. By mood disorders or related disorders is meant the following disorders as defined in the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV) taxonomy (DSM-IV codes in parenthesis):- mood disorders (296.XX, 300.4, 311, 301.13, 295.70), schizophrenia and related disorders (295.XX, 297.1, 298.8, 297.3, 298.9), anxiety disorders (300.XX, 309.81, 308.3), adjustment disorders (309.XX) and personality disorders (codes 301.XX).

The methods of the invention are particularly exemplified in relation to genetic factors associated with a family of mood disorders known as Bipolar (BP) spectrum disorders.

Bipolar disorder (BP) is a severe psychiatric condition that is characterized by disturbances in mood, ranging from an extreme state of elation (mania) to a severe state of dysphoria (depression). Two types of bipolar illness have been described: type I BP illness (BPI) is characterized by major depressive episodes alternated with phases of mania, and type II

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BP illness (BPII), characterized by major depressive episodes alternating with phases of hypomania. Relatives of BP probands have an increased risk for BP, unipolar disorder (patients only experiencing depressive episodes; UP), cyclothymia (minor depression and hypomania episodes; CY) as well as for schizoaffective disorders of the manic (SA_m) and depressive (SA_d) type. Based on these observations BP, CY, UP and SA are classified as BP spectrum disorders.

The involvement of genetic factors in the etiology of BP spectrum disorders was suggested by family, twin and adoption studies (Tsuang and Faraone (1990), The Genetics of Mood Disorders, Baltimore, The John Hopkins University Press). However, the exact pattern of transmission is unknown. In some studies, complex segregation analysis supports the existence of a single major locus for BP (Spence et al. (1995), Am J. Med. Genet (Neuropsych. Genet.) 60 pp 370-376). Other researchers propose a liability-threshold-model, in which the liability to develop the disorder results from the additive combination of multiple genetic and environmental effects (McGuffin et al. (1994), Affective Disorders; Seminars in Psychiatric Genetics Gaskell, London pp 110-127).

Due to the complex mode of inheritance, parametric and nonparametric linkage strategies are applied in families in which BP disorder appears to be transmitted in a Mendelian fashion. Early linkage findings on chromosomes 11p15 (Egeland et al. (1987), Nature 325 pp 783-787) and Xq27-q28 (Mendlewicz et al. (1987) The Lancet 1 pp 1230 -1232; Baron et al. (1987) Nature 326 pp 289-292) have been controversial and could initially not be replicated (Kelsoe et al. (1989) Nature 242 pp 238-243; Baron et al. (1993) Nature Genet 3 pp 49-55). With the development of a

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human genetic map saturated with highly polymorphic markers and the continuous development of data analysis techniques, numerous new linkage searches were started. In several studies, evidence or
5 suggestive evidence for linkage to particular regions on chromosomes 4, 12, 18, 21 and X was found (Blackwood et al. (1996) Nature Genetics 12 pp 427-430, Craddock et al. (1994) Brit J. Psychiatry 164 pp 355-358, Berrettini et al. (1994), Proc Natl Acad Sci
10 USA 91 pp 5918-5921, Straub et al. (1994) Nature Genetics 8 pp 291-296 and Pekkariinen et al. (1995) Genome Research 5 pp 105-115). In order to test the validity of the reported linkage results, these findings have to be replicated in other, independent
15 studies.

Recently, linkage of bipolar disorder to the pericentromeric region on chromosome 18 was reported (Berrettini et al. 1994). Also a ring chromosome 18 with break-points and deleted regions at 18pter-p11
20 and 18q23-qter was reported in three unrelated patients with BP illness or related syndromes (Craddock et al. 1994). The chromosome 18p linkage was replicated by Stine et al. (1995) Am J Hum Genet 57 pp 1384-1394, who also reported suggestive evidence
25 for a locus on 18q21.2-q21.32 in the same study. Interestingly, Stine et al. observed a parent-of-origin effect: the evidence of linkage was the strongest in the paternal pedigrees, in which the proband's father or one of the proband's father's sibs
30 is affected.

In an independent replication study, the present inventors tested linkage with chromosome 18 markers in
10 Belgian families with a bipolar proband. To localize causative genes the linkage analysis or
35 likelihood method was used in these families. This

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method studies within a family the segregation of a defined disease phenotype with that of polymorphic genetic markers distributed in the human genome. The likelihood ratio of observing cosegregation of the disease and a genetic marker under linkage versus no linkage is calculated and the log of this ratio or the log of the odds is the LOD score statistic z . A LOD score of 3 (or likelihood ratio of 1000 or greater) is taken as significant statistical evidence for linkage.

In the inventors' study no evidence for linkage to the pericentromeric regions was found, but in one of the families, MAD31, a Belgian family of a BPII proband, suggestive linkage was found with markers located at 18q21.33-q23 (De bruyn et al. (1996) Biol Psychiatry 39 pp 679-688). Multipoint linkage analysis gave the highest LOD score in the interval between STR (Short Tandem Repeats) polymorphisms D18S51 and D18S61, with a maximum multipoint LOD score of +1.34. Simulation studies indicated that this LOD score is within the range of what can be expected for a linked marker given the information available in the family. Likewise, an affected sib-pair analysis also rejected the null-hypothesis of nonlinkage for several of the markers tested. Two other groups also found evidence for linkage of bipolar disorder to 18q (Freimer et al. (1996) Nature Genetics 12 pp 436-441, Coon et al. (1996) Biol Psychiatry 39 pp 689 to 696). Although the candidate regions in the different studies do not entirely overlap, they all suggest the presence of a susceptibility locus at 18q21-q23.

The inventors have now carried out further investigations into the 18q chromosomal region in family MAD31. By analysis of cosegregation of bipolar disease in MAD31 with twelve STR polymorphic markers previously located between the aforementioned markers

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D18S51 and D18S61 and subsequent LOD score analysis as described above, the inventors have further refined the candidate region of chromosome 18 in which a gene associated with mood disorders such as bipolar
5 spectrum disorders may be located and have constructed a physical map. The region in question may thus be used to locate, isolate and sequence a gene or genes which influences psychiatric health and mood.

The inventors have also constructed a YAC (yeast
10 artificial chromosome) contig map of the candidate region to determine the relative order of the twelve STR markers mapped by the cosegregational analysis and they have identified seven clones from the YAC library incorporating the candidate region.

15 A number of procedures can be applied to the identified YAC clones and, where applicable, to the DNA of an individual afflicted with a mood disorder as defined herein, in the process of identifying and characterising the relevant gene or genes. For
20 example, the inventors have used YAC clones spanning the region of interest in chromosome 18 to identify by CAG or CTG fragmentation novel genes that are allegedly involved in the manifestation of mood disorders or related disorders.

25 Other procedures can also be applied to the said YAC clones to identify candidate genes as discussed below.

Once candidate genes have been identified it is possible to assess the susceptibility of an individual
30 to a mood disorder or related disorder by detecting the presence of a polymorphism associated with a mood disorder or related disorder in such genes.

Accordingly, in a first aspect the present
35 invention comprises the use of an 8.9 cM region of

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human chromosome 18q disposed between polymorphic markers D18S68 and D18S979 or a fragment thereof for identifying at least one human gene, including mutated and polymorphic variants thereof, which is associated with mood disorders or related disorders as defined above. As will be described below, the present inventors have identified this candidate region of chromosome 18q for such a gene, by analysis of cosegregation of bipolar disease in family MAD31 with 12 STR polymorphic markers previously located between D18S51 and D18S61 and subsequent LOD score analysis.

In a second aspect the invention comprises the use of a YAC clone comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61 for identifying at least one human gene, including mutated or polymorphic variants thereof, which is associated with mood disorders or related disorders as defined above. D18S60 is close to D18S51 so the particular YAC clones for use are those which have an artificial chromosome spanning the candidate region of human chromosome 18q between polymorphic markers D18S51 and D18S61 as identified by the present inventors in their earlier paper (De bruyn *et al.* (1996)).

Particular YACs covering the candidate region which may be used in accordance with the present invention are 961.h.9, 942.c.3, 766.f.12, 731.c.7, 907.e.1, 752-g-8 and 717.d.3, preferred ones being 961.h.9, 766.f.12 and 907.e.1 since these have the minimum tiling path across the candidate region. Suitable YAC clones for use are those having an artificial chromosome spanning the refined candidate region between D18S68 and D18S979.

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There are a number of methods which can be applied to the candidate regions of chromosome 18q as defined above, whether or not present in a YAC, to identify a candidate gene or genes associated with mood disorders or related disorders. For example, it has previously been demonstrated that an apparent association exists between the presence of trinucleotide repeat expansions (TRE) in the human genome and the phenomenon of anticipation of mood disorders (Lindblad et al. (1995), Neurobiology of Disease 2: 55-62 and O'Donovan et al. (1995), Nature Genetics 10: 380-381).

Accordingly, in a third aspect the present invention comprises a method of identifying at least one human gene, including mutated and polymorphic variants thereof, which is associated with a mood disorder or related disorder as defined herein which comprises detecting nucleotide triplet repeats in the region of human chromosome 18q disposed between polymorphic markers D18S68 and D18S979.

An alternative method of identifying said gene or genes comprises fragmenting a YAC clone comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61, for example one or more of the seven aforementioned YAC clones, and detecting any nucleotide triplet repeats in said fragments. Nucleic acid probes comprising at least 5 and preferably at least 10 CTG and/or CAG triplet repeats are a suitable means of detection when appropriately labelled. Trinucleotide repeats may also be determined using the known RED (repeat expansion detection) system (Shalling et al.(1993), Nature Genetics 4 pp 135-139).

In a fourth embodiment the invention comprises a

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method of identifying at least one gene, including mutated and polymorphic variants thereof, which is associated with a mood disorder or related disorder and which is present in a YAC clone spanning the region of human chromosome 18q between polymorphic markers D18S60 and D18S61, the method comprising the step of detecting the expression product of a gene incorporating nucleotide triplet repeats by use of an antibody capable of recognising a protein with an amino acid sequence comprising a string of at least 8, but preferably at least 12, continuous glutamine residues. Such a method may be implemented by subcloning YAC DNA, for example from the seven aforementioned YAC clones, into a human DNA expression library. A preferred means of detecting the relevant expression product is by use of a monoclonal antibody, in particular mAB 1C2, the preparation and properties of which are described in International Patent Application Publication No WO 97/17445.

As will be described in detail below, in order to identify candidate genes containing triplet repeats, the inventors have carried out direct CAG or CTG fragmentation of YACs 961.h.9, 766.f.12 and 907.e.1, comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61, and have identified a number of sequences containing CAG or CTG repeats, whose abnormal expansion may be involved in genetic susceptibility to a mood disorder or related disorder.

Accordingly, in a fifth aspect, the invention provides a nucleic acid comprising the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a.

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In a further aspect, the invention provides a protein comprising an amino acid sequence encoded by the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a.

5

In yet a further aspect the invention provides a mutated nucleic acid comprising a sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats.

10

Also provided by the invention is a mutated protein comprising an amino acid sequence encoded by a sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats.

15

It is to be understood that the invention also contemplates nucleotide sequences having at least 75% and preferably at least 80% homology with any of the sequences described above and having functional identity with any of said sequences. The homology is calculated as described by Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, Karlin et al. (1990) Proc Natl Acad Sci USA 87: 2264-68 and Karlin et al. (1993) Proc Natl Acad Sci USA 90: 5873-5877. Also contemplated are amino acid sequences which differ from the above described sequences only in conservative amino acid changes. Suitable changes are well known to those skilled in the art.

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Knowledge of the sequences described above can be used to design assays to determine the genetic susceptibility of an individual to a mood disorder or

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- 10 -

related disorder.

Accordingly, in a further aspect the invention provides a method for determining the susceptibility of an individual to a mood disorder or related disorder which comprises the steps of:

5

a) obtaining a DNA sample from said individual;

10

b) providing primers suitable for the amplification of a nucleotide sequence comprised in the sequence shown in any one of Figures 15a, 16a, 17a or 18a said primers flanking the trinucleotide repeats comprised in said sequence;

15

c) applying said primers to the said DNA sample and carrying out an amplification reaction;

20

d) carrying out the same amplification reaction on a DNA sample from a control individual; and

25

e) comparing the results of the amplification reaction for the said individual and for the said control individual;

wherein the presence of an amplified fragment from said individual which is bigger in size from that of said control individual is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

30

By control individual is meant an individual who is not affected by a mood disorder or related disorder and does not have a family history of mood disorders or related disorders.

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Preferable primers to use in this method are those shown in Figure 15b, 16b, 17b or 18b but other suitable primers may be utilised.

5 In a further aspect the invention provides a method of determining the susceptibility of an individual to a mood disorder or related disorder which method comprises the steps of :

10 a) obtaining a protein sample from said individual; and

 b) detecting the presence of a protein comprising an amino acid sequence encoded by a
15 sequence of nucleotides which differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a, or 18a only in the extent of trinucleotide repeats

20 wherein the presence of said protein is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.
 Preferably, the foresaid protein is detected by utilising an antibody that is capable of recognising a
25 string of at least 8 continuous glutamines as, for example, the mAB 1C2 antibody.

 The nucleic acids molecules according to the invention may be advantageously included in an
30 expression vector, which may be introduced into a host cell of prokaryotic or eukaryotic origin. Suitable expression vectors include plasmids, which may be used to express foreign DNA in bacterial or eukaryotic host cells, viral vectors, yeast artificial chromosomes or
35 mammalian artificial chromosomes. The vector may be

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transfected or transformed into host cells using suitable methods known in the art such as, for example, electroporation, microinjection, infection, lipoinfection and direct uptake. Such methods are described in more detail, for example, by Sambrook et al., "Molecular Cloning: A Laboratory Manual", 2nd ed. (1989) and by Ausbel et al. "Current Protocols in Molecular Biology", (1994).

Also provided by the invention is a host cell, tissue or organism comprising the expression vector according to the invention. The invention further provides a transgenic host cell, tissue or organism comprising a transgene capable of encoding the proteins of the invention, which may comprise a genomic DNA or a cDNA. The transgene may be present in the transgenic host cell, tissue or organism either stably integrated into the genome or in an extra chromosomal state.

A nucleic acid molecule comprising a nucleotide sequence shown in any one of Figures 15a, 16a, 17a or 18a as well as the protein encoded by it may be therapeutically used in the treatment of mood disorders or related disorders in patients which present a trinucleotide repeat expansion (TRE) in at least one of the foresaid sequences.

Accordingly, in another of its aspects the invention provides the above described nucleic acid molecules and proteins for use as medicaments for the treatment of individuals with a mood disorder or related disorder. Preferably, the nucleic acid or the protein is present in an appropriate carrier or delivery vehicle. As an example, the nucleic acid inserted into a vector, for example a plasmid or a

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viral vector, may be transfected into a mammalian cell such as a somatic cell or a mammalian germ line cell, as described above. The cell to be transfected can be present in a biological sample obtained from the patient, for example blood or bone marrow, or can be obtained from cell culture. After transfection the sample may be returned or readministered to a patient according to methods known to those practised in the art, for example, methods as described in Kasid et al., Proc. Natl. Acad. Sci. USA (1990) 87:473; Rosenberg et al. (1990) New Eng. J. Med. 323: 570 ; Williams et al. (1994) Nature 310: 476; Dick et al. (1985) Cell 42:71; Keller et al. (1985) Nature 318: 149 and Anderson et al. (1994) US Patent N. 5,399,346.

There are a number of viral vectors known to those skilled in the art which can be used to introduce the nucleic acid into mammalian cells, for example retroviruses, parvoviruses, coronaviruses, negative strand RNA viruses such as picornaviruses or alphaviruses and double stranded DNA viruses including adenoviruses, herpesviruses such as Herpes Simplex virus types 1 and 2, Epstein-Barr virus or cytomegalovirus and poxviruses such as vaccinia fowlpox or canarypox. Other viruses include, for example, Norwalk viruses, togaviruses, flaviviruses, reoviruses, papovaviruses, hepadnaviruses and hepatitis viruses.

A preferred method to introduce nucleic acid that encodes the desired protein into cells is through the use of engineered viral vectors. These vectors provide a means to introduce nucleic acids into cycling and quiescent cells and have been modified to reduce cytotoxicity and to improve genetic stability. The preparation and use of engineered Herpes simplex virus type 1 (D.M. Krisky, et al. (1997) Gene Therapy

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4(10): 1120-1125), adenoviral (A. Amalfitano, et al.(1998) Journal of Virology 72(2):926-933), attenuated lentiviral (R. Zufferey, et al., Nature Biotechnology (1997) 15(9):871-875) and

5 adenoviral/retroviral chimeric (M. Feng, et al, Nature Biotechnology (1997) 15(9):866-870) vectors are known to the skilled artisan.

The protein may be administered using methods known in the art. For example, the mode of
10 administration is preferably at the location of the target cells. The administration can be by injection. Other modes of administration (parenteral, mucosal, systemic, implant, intraperitoneal, etc.) are generally known in the art. The agents can,
15 preferably, be administered in a pharmaceutically acceptable carrier, such as saline, sterile water, Ringer's solution and isotonic sodium chloride solution.

20 In yet another of its aspects the invention provides assay methods for identifying compounds that are able to enhance or inhibit the expression of the proteins of the invention. These assays can be conducted, for example, by transfecting a nucleic acid
25 of the invention into host cells and then comparing the levels of mRNA transcript or the levels of protein expressed from said nucleic acids in the presence or absence of the compound.

Different methods, well known to those skilled in the
30 art can be employed in order to measure transcription or expression levels.

Alternatively, it is possible to identify compounds that modulate transcription by using a reporter gene assay of the type well known in the art. In such an
35 assay a reporter plasmid is constructed in which the

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promoter of a gene, whose levels of transcription are to be monitored, is positioned upstream of a gene capable of expressing a reporter molecule. The reporter molecule is a molecule whose level of
5 expression can be easily detected and may be either the transcript of the reporter gene or a protein with characteristics that allow it to be detected. For example, the molecule may be a fluorescent protein such as green fluorescent protein (GFP).

10 Compound assays may be conducted by introducing the reporter plasmid described above into an appropriate host cell and then measuring the amount of reporter molecule expressed in the presence or absence of the compound to be tested.

15 The invention also relates to compounds identified by the above mentioned methods.

Further embodiments of the present invention
20 relate to methods of identifying the relevant gene or genes which involve the sub-cloning of YAC DNA as defined above into vectors such as BAC (bacterial artificial chromosome) or PAC (P1 or phage artificial chromosome) or cosmid vectors such as exon-trap cosmid
25 vectors. The starting point for such methods is the construction of a contig map of the region of human chromosome 18q between polymorphic markers D18S60 and D18S61. To this end the present inventors have sequenced the end regions of the fragment of human DNA
30 in each of the seven aforementioned YAC clones and these sequences are disclosed herein. Following subcloning of YAC DNA into other vectors as described above, probes comprising these end sequences or portions thereof, in particular those sequences shown
35 in Figures 1 to 11 herein, together with any known

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sequenced tagged site (STS) in this region, as described in the YAC clone contig shown herein, as can be used to detect overlaps between said subclones and a contig map can be constructed. Also the known
5 sequences in the current YAC contig can be used for the generation of contig map subclones.

One route by which a gene or genes which is associated with a mood disorder or associated disorder
10 can be identified is by use of the known technique of exon trapping.

This is an artificial RNA splicing assay, most often making use in current protocols of a specialized exon-trap cosmid vector. The vector contains an
15 artificial minigene consisting of a segment of the SV40 genome containing an origin of replication and a powerful promoter sequence, two splicing-competent exons separated by an intron which contains a multiple cloning site and an SV40 polyadenylation site.

20 The YAC DNA is subcloned in the exon-trap vector and the recombinant DNA is transfected into a strain of mammalian cells. Transcription from the SV40 promoter results in an RNA transcript which normally splices to include the two exons of the minigene. If
25 the cloned DNA itself contains a functional exon, it can be spliced to the exons present in the vector's minigene. Using reverse transcriptase a cDNA copy can be made and using specific PCR primers, splicing events involving exons of the insert DNA can be
30 identified. Such a procedure can identify coding regions in the YAC DNA which can be compared to the equivalent regions of DNA from a person afflicted with a mood disorder or related disorder to identify the relevant gene.

35 Accordingly, in a further aspect the invention

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comprises a method of identifying at least one human gene, including mutated variants and polymorphisms thereof, which is associated with a mood disorder or related disorder which comprises the steps of:

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(a) transfecting mammalian cells with exon trap cosmid vectors prepared and mapped as described above;

10 (b) culturing said mammalian cells in an appropriate medium;

(c) isolating RNA transcripts expressed from the SV40 promoter;

15 (d) preparing cDNA from said RNA transcripts;

20 (e) identifying splicing events involving exons of the DNA subcloned into said exon trap cosmid vectors to elucidate positions of coding regions in said subcloned DNA;

25 (f) detecting differences between said coding regions and equivalent regions in the DNA of an individual afflicted with said mood disorder or related disorder; and

(g) identifying said gene or mutated or polymorphic variant thereof which is associated with said mood disorder or related disorders.

30

As an alternative to exon trapping the YAC DNA may be subcloned into BAC, PAC, cosmid or other vectors and a contig map constructed as described above. There are a variety of known methods available
35 by which the position of relevant genes on the

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subcloned DNA can be established as follows:

(a) cDNA selection or capture (also called direct selection and cDNA selection): this method involves the forming of genomic DNA/cDNA heteroduplexes by hybridizing a cloned DNA (e.g. an insert of a YAC DNA), to a complex mixture of cDNAs, such as the inserts of all cDNA clones from a specific (e.g. brain) cDNA library. Related sequences will hybridize and can be enriched in subsequent steps using biotin-streptavidine capturing and PCR (or related techniques);

(b) hybridization to mRNA/cDNA: a genomic clone (e.g. the insert of a specific cosmid) can be hybridized to a Northern blot of mRNA from a panel of culture cell lines or against appropriate (e.g. brain) cDNA libraries. A positive signal can indicate the presence of a gene within the cloned fragment;

(c) CpG island identification: CpG or HTF islands are short (about 1 kb) hypomethylated GC-rich (> 60%) sequences which are often found at the 5' ends of genes. CpG islands often have restriction sites for several rare-cutter restriction enzymes. Clustering of rare-cutter restriction sites is indicative of a CpG island and therefore of a possible gene. CpG islands can be detected by hybridization of a DNA clone to Southern blots of genomic DNA digested with rare-cutting enzymes, or by island-rescue PCR (isolation of CpG islands from YACs by amplifying sequences between islands and neighbouring Alu-repeats);

(d) zoo-blotting: hybridizing a DNA clone (e.g.

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the insert of a specific cosmid) at reduced stringency against a Southern blot of genomic DNA samples from a variety of animal species. Detection of hybridization signals can suggest conserved sequences, indicating a possible gene.

Accordingly, in a further aspect the invention comprises a method of identifying at least one human gene including mutated and polymorphic variants thereof which is associated with a mood disorder or related disorder which comprises the steps of:

(a) subcloning the YAC DNA as described above into a cosmid, BAC, PAC or other vector;

(b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other sequenced tagged site (STS) in this region as in the YAC clone contig described herein, or part thereof consisting of not less than 14 contiguous bases or the complement thereof, to detect overlaps amongst the subclones and construct a map thereof;

(c) identifying the position of genes within the subcloned DNA by one or more of CpG island identification, zoo-blotting, hybridization of the subcloned DNA to a cDNA library or a Northern blot of mRNA from a panel of culture cell lines;

(d) detecting differences between said genes and equivalent region of the DNA of an individual afflicted with a mood disorder or related disorder; and

(e) identifying said gene which is associated

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with said mood disorders or related disorders.

If the cloned YAC DNA is sequenced, computer analysis can be used to establish the presence of relevant genes. Techniques such as homology searching and exon prediction may be applied.

Once a candidate gene has been isolated in accordance with the methods of the invention more detailed comparisons may be made between the gene from a normal individual and one afflicted with a mood disorder such as a bipolar spectrum disorder. For example, there are two methods, described as "mutation testing", by which a mutation or polymorphism in a DNA sequence can be identified. In the first the DNA sample may be tested for the presence or absence of one specific mutation but this requires knowledge of what the mutation might be. In the second a sample of DNA is screened for any deviation from a control (normal) DNA. This latter method is more useful for identifying candidate genes where a mutation is not identified in advance.

In addition, the following techniques may be further applied to a gene identified by the above-described methods to identify differences between genes from normal or healthy individuals and those afflicted with a mood disorder or related disorder:

(a) Southern blotting techniques: a clone is hybridized to nylon membranes containing genomic DNA digested with different restriction enzymes of patients and healthy individuals. Large differences between patients and healthy individuals can be visualized using a radioactive labelling protocol;

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(b) heteroduplex mobility in polyacrylamide gels: this technique is based on the fact that the mobility of heteroduplexes in non-denaturing polyacrylamide gels is less than the mobility of homoduplexes. It is most effective for fragments under 200 bp;

(c) single-strand conformational polymorphism analysis (SSCP or SSCA): single stranded DNA folds up to form complex structures that are stabilized by weak intramolecular bonds. The electrophoretic mobilities of these structures on non-denaturing polyacrylamide gels depends on their chain lengths and on their conformation;

(d) chemical cleavage of mismatches (CCM): a radiolabelled probe is hybridized to the test DNA, and mismatches detected by a series of chemical reactions that cleave one strand of the DNA at the site of the mismatch. This is a very sensitive method and can be applied to kilobase-length samples;

(e) enzymatic cleavage of mismatches: the assay is similar to CCM, but the cleavage is performed by certain bacteriophage enzymes.

(f) denaturing gradient gel electrophoresis: in this technique, DNA duplexes are forced to migrate through an electrophoretic gel in which there is a gradient of increasing amounts of a denaturant (chemical or temperature). Migration continues until the DNA duplexes reach a position on the gel wherein the strands melt and separate, after which the denatured DNA does not migrate much further. A single base pair difference between a normal and a mutant DNA duplex is sufficient to cause them to migrate to

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different positions in the gel;

(g) direct DNA sequencing.

5 It will be appreciated that with respect to the
methods described herein, in the step of detecting
differences between coding regions from the YAC and
the DNA of an individual afflicted with a mood
disorder or related disorder, the said individual may
10 be anybody with the disorder and not necessary a
member of family MAD31.

15 In accordance with further aspects the present
invention provides an isolated human gene and variants
thereof associated with a mood disorder or related
disorder and which is obtainable by any of the above
described methods, an isolated human protein encoded
by said gene and a cDNA encoding said protein.

20 In the experimental report which follows
reference will be made to the following figures:

25 FIGURE 1 shows a sequence of nucleotides which is
the left arm end-sequence of YAC 766_f.12;

 FIGURE 2 shows a sequence of nucleotides which is
a right arm end-sequence of YAC 766_f.12;

30 FIGURE 3 shows a sequence of nucleotides which is
a left arm end-sequence of YAC 717_d.3;

 FIGURE 4 shows a sequence of nucleotides which is
a right arm end-sequence of YAC 717_d.3;

35 FIGURE 5 shows a sequence of nucleotides which is

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a right arm end-sequence of YAC 731.c.7;

FIGURE 6 shows a sequence of nucleotides which is
a left arm end-sequence of YAC 752.g.8;

5

FIGURE 7 shows a sequence of nucleotides which is
a left arm end-sequence of YAC 942.c.3;

FIGURE 8 shows a sequence of nucleotides which is
10 a right arm end-sequence of YAC 942.c.3;

FIGURE 9 shows a sequence of nucleotides which is
a left arm end-sequence of YAC 961.h.9;

15 FIGURE 10 shows a sequence of nucleotides which
is a right arm end-sequence of YAC 961.h.9;

FIGURE 11 shows a sequence of nucleotides which
is a left arm end-sequence of YAC 907.e.1;

20

FIGURE 12 shows a pedigree of family MAD31;

FIGURE 13 shows the haplotype analysis for family
MAD13. Affected individuals are represented by filled
25 diamonds, open diamonds represent individuals who were
asymptomatic at the last psychiatric evaluation. Dark
gray bars represent markers for which it cannot be
deduced if they are recombinant; and

30 FIGURE 14 shows the YAC contig map of the region
of human chromosome 18 between the polymorphic markers
D18560 and D18561. Black lines represent positive
hits. YACs are not drawn to scale.

35 FIGURE 15 shows (a) a CAG repeat (in bold) and

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surrounding nucleotide sequence isolated from YAC
961_h_9. The sequence in italics is derived from End
Rescue of the fragmented YAC. (b) PCR primers that can
be used to determine the extent of trinucleotide
5 repeats in the sequence.

FIGURE 16 shows (a) a CAG repeat (in bold) and
surrounding nucleotide sequence isolated from YAC
766_f_12. The sequence in italics is derived from End
10 Rescue of the fragmented YAC. (b) PCR primers that can
be used to determine the extent of trinucleotide
repeats in the sequence.

FIGURE 17 shows (a) a CAG repeat (in bold) and
15 surrounding nucleotide sequence isolated from YAC
766_f_12. The sequence in italics is derived from End
Rescue of the fragmented YAC. (b) PCR primers that can
be used to determine the extent of trinucleotide
repeats in the sequence.

FIGURE 18 shows (a) a CTG repeat (in bold) and
surrounding nucleotide sequence isolated from YAC
907_e_1. The sequence in italics is derived from End
Rescue of the fragmented YAC. (b) PCR primers that can
25 be used to determine the extent of trinucleotide
repeats in the sequence.

Experimental 1

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(a) Family Data

Clinical diagnoses in MAD31, a Belgian family with a
BP11 proband were described in detail in De bruyn et
35 al 1996. In that study only the 15 family members who

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were informative for linkage analysis were selected for additional genotyping. The different clinical diagnoses in the family were as follows:

- 1 BPI, 2 BPII, 2UP, 4 Major depressive disorder (MDD),
5 1 SAm and 1 SAd.

The pedigree of the MAD31 family is shown in Figure 12.

(b) Genotyping of Family Members

10

All short tandem repeat (STR) genetic markers are di- or tetranucleotide repeat polymorphisms. Information concerning the genetic markers used in this study was obtained from several sources on the internet: Genome

15 DataBase (GDB, <http://gdbwww.gdb.org/>), GenBank

(<http://www.ncbi.nlm.nih.gov/>), Cooperative Human Linkage Center (CHLC, <http://www.chlc.org/>), Eccles Institute of Human Genetics (EIHG, <http://www.genetics.utah.edu/>) and Généthon

20 (<http://www.genethon.fr/>). Standard PCR was performed in a 25 µl volume containing 100 ng genomic DNA, 200 mM of each dNTP, 1.25 mM MgCl₂, 30 pmol of each primer and 0.2 units Goldstar DNA polymerase

(Eurogentec). One primer was end-labelled before PCR with [gamma-³²P]ATP and T4 polynucleotide kinase. After an initial denaturation step at 94°C for 2 min, 27

cycles were performed at 94°C for 1 min, at the appropriate annealing temperature for 1.5 min and

extension at 72°C for 2 min. Finally, an additional elongation step was performed at 72°C for 5 min. PCR products were detected by electrophoresis on a 6% denaturing polyacrylamide gel and by exposure to an X-ray sensitive film. Successfully analysed STSS, STRs and ESTs covering the refined candidate region are

35 fully described herein on pages 36 to 54.

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(c) Lod score analysis.

Two-point lod scores were calculated for 3 different disease models using Fastlink 2.2. (Cottingham et al. 1993). For all models, a disease gene frequency of 1% and a phenocopy rate of 1/1000 was used. Model 1 included all patients and unaffected individuals with the latter individuals being assigned to a disease penetrance class depending on their age at examination. The 9 age-dependent penetrance classes as described by De bruyn et al (1996) were multiplied by a factor 0.7 corresponding to a reduction of the maximal penetrance of 99% to 70% for individuals older than 60 years (Ott 1991). Model 2 is similar to model 1, but patients were assigned a diagnostic stability score, calculated based on clinical data such as the number of episodes, the number of symptoms during the worst episode and history of treatment (Rice et al. 1987, De bruyn et al. 1996). Model 3 is as model 1 but includes only patients.

(d) Construction of the YAC contig - protocols

Growing of YACs and extraction of YAC DNA was done according to standard protocols (Silverman, 1995). For the construction of the YAC-contig spanning the chromosome 18q candidate region, the data of the physical map based on sequence tagged sites (STSs) (Hudson et al. 1995) was consulted on the Whitehead Institute (WI) Internet site (<http://www-genome.wi.mit.edu/>). CEPH mega-YACs were obtained from the YAC Screening Centre Leiden (YSCL, the Netherlands) and from CEPH (Paris, France). The YACs were analyzed for the presence of STSs and STRs, previously located between D18S51 and D18S61, by

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touchdown PCR amplification. Information on the STSs/STRs was obtained from the WI, GDB, Généthon, CHLC and GenBank sites on the Internet. Thirty PCR cycles consisted of: denaturation at 94°C for 1 min, annealing (2 cycles for each temperature) starting from 65°C and decreasing to 51°C for 1.5 min and extension at 72°C for 2 min. This was followed by 10 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1.5 min and extension at 72°C for 2 min. A final extension step was performed for 10 min at 72°C. Amplified products were visualised by electrophoresis on a 1% TBE agarose gel and ethidium bromide staining.

(e) Ordering of the STR markers.

Twelve STR markers, previously located between D18S51 and D18S61, were tested for cosegregation with bipolar disease in family MAD31. The parental haplotypes were reconstructed from genotype information of the siblings in family MAD31 and minimalizing the number of possible recombinants. The result of this analysis is shown in Figure 13. The father was not informative for 3 markers, the mother was not informative for 5 markers. Haplotypes in family MAD31 suggested the following order for the STR markers analysed: cen-[S51-S68-S346]-[S55-S969-S1113-S483-S465]-[S876-S477]-S979-[S466-S817-S61]-tel. The order relative to each other of the markers between brackets could not be inferred from our haplotype data. The marker order in family MAD31 was compared with the marker order obtained using different mapping techniques and the results shown in Table 1 below.

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Table 1. Comparison of the order of the markers within the 18q candidate region for bipolar disorder, among several maps.

5	Marker*	Genetic maps		Radiation hybrid map
		G��n��thon	Marshfield	(Giacalone et al. 1996)
	D18S51		(-)3.4cM	(-)27.9 cR
10	D18S68	0 cM	0 cM	0 cR
	D18S346		5.3 cM	52.2 cR
	D18S55	0.1 cM	0 cM	72.5 cR
15	D18S969		0.6 cM	
	D18S1113	0.7 cM		
	D18S483	2.5 cM	3.2 cM	88 cR
20	D18S465	4.5 cM	5.3 cM	101.3 cR
	D18S876			
	D18S477	4.4 cM	5.3 cM	166.4 cR
25	D18S979		8.9 cM	
	D18S466	7.6 cM	11.1 cM	212.4 cR
	D18S61	8.4 cM	11.8 cM	249.5 cR
30	D18S817		5.3 cM	260.6 cR

* Order according to haplotyping results in family MAD31.

(-) Marker is located proximal of D18S68.

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- 29 -

D18S68, common to all 3 maps, was taken as the map anchor point, and the genetic distance in cM or cR of the other markers relative to D18S68 are given. The marker order is in good agreement with the order of the markers on the recently published chromosome 18 radiation hybrid map (Giacalone et al. (1996) Genomics 37:9-18) and the WI YAC-contig map (<http://www-genome.wi.mit.edu/>). However, a few discrepancies with other maps were observed. The only discrepancy with the Généthon genetic map is the reversed order of D18S465 and D18S477. Two discrepancies were observed with the Marshfield map (<http://www.marshmed.org/genetics/>). The present inventors mapped D18S346 above D18S55 based on maternal haplotypes, but on the Marshfield maps D18S346 is located between D18S483 and D18S979. The inventors also placed D18S817 below D18S979, but on the Marshfield map this marker is located between D18S465 and D18S477. However, the location of D18S346 and D18S817 is in agreement with the chromosome 18 radiation hybrid map of Giacalone et al. (1996). One discrepancy was also observed with the WI radiation hybrid map (<http://www-genome.wi.mit.edu/>), in which D18S68 was located below D18S465. However, the inventors as well as other maps placed this marker above D18S55.

(f) Lod score analysis and refinement of the candidate region.

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Lod score analysis gave positive results with all markers, confirming the previous observation that 18q21.33-q23 is implicated in BP disease, at least in family MAD31 (De bruyn et al. 1996). Summary statistics of the lod score analysis under all models

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are given in table 2 below.

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Table 2. Summary statistics of the two-point lod scores in MAD31.

Marker	Model 1			Model 2			Model 3		
	Z at $\theta=0.0$	Zmax	θ_{\max}	Z at $\theta=0.0$	Zmax	θ_{\max}	Z at $\theta=0.0$	Zmax	θ_{\max}
D18S51	-0.19	0.73	0.1	0.94	0.94	0.01	0.08	0.54	0.1
D18S68	-0.19	0.73	0.1	0.94	0.94	0.01	0.07	0.55	0.1
D18S346	-0.19	0.73	0.1	0.94	0.94	0.01	0.07	0.55	0.1
D18S969	1.40	1.40	0.0	1.27	1.27	0.0	1.20	1.20	0.0
D18S1113	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S876	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S477	2.01	2.01	0.0	1.87	1.87	0.0	1.77	1.77	0.0
D18S979	-0.18	0.77	0.1	1.08	1.08	0.0	0.08	0.54	0.0
D18S817	-0.19	0.73	0.1	1.08	1.08	0.0	0.07	0.55	0.1
D18S61	-0.21	0.73	0.1	1.08	1.08	0.0	0.07	0.54	0.1

D18S55, D18S483, D18S465 and D18S466 were not informative.

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The highest two-point lod score (+2.01 at $\theta=0.0$) was obtained with markers D18S1113, D18S876 and D18S477 under model 1 in the absence of recombinants (table 2). In model 1, all individuals with a BP spectrum disorder are considered affected and fully contributing to the linkage analysis.

Before the fine mapping the candidate region was flanked by D18S51 and D18S61, which are separated by a genetic distance of 15.2 cM on the Marshfield map or 13.1 cM on the Généthon map. The informative recombinants with D18S51 and D18S61 were observed in 2 affected individuals (II.10 and II.11 in Fig. 13). However, since no other markers were tested within the candidate region it was not known whether these individuals actually shared a region identical-by-descent (IBD). The additional genetic mapping data now indicate that all affected individuals are sharing alleles at D18S969, D18S1113, D18S876 and D18S477 (Fig. 13, boxed haplotype). Also, alleles from markers D18S483 and D18S465 are probably IBD, but these markers were not informative in the affected parent I.1. Obligate recombinants were observed with the STR markers D18S68, D18S346, D18S979 and D18S817 (Table 2, fig. 13) Since discrepancies between different maps were observed for the locations of D18S346 and D18S817, the present inventors used D18S68 and D18S979 to redefine the candidate region for BP disease. The genetic distance between these 2 markers is 8.9 cM based on the Marshfield genetic map (<http://www.marshmed.org/genetics/>).

(g) Construction of the YAC contig.

According to the WI integrated map 56 CEPH megaYACs are located in the initial candidate region

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contained between D18S51 and D18S61 (Chumakov et al. (1995) Nature 377 Suppl., De bruyn et al. (1996)). From these YACs, those were selected that were located in the region between D18S60 and D18S61. D18S51 is not presented on the WI map, but is located close to D18S60 according to the Marshfield genetic map (<http://www.marshmed.org/genetics/>). To limit the number of potential chimaeric YACs, YACs were eliminated that were also positive for non-chromosome 18 STSs. As such, 25 YACs were selected (see Figure 14), and placed in a contig based on the technique of YAC contig mapping, i.e. sequences from sequence tagged sites (STSs), simple tandem repeats (STRs) and expressed sequenced tags (ESTs), known to map between D18S60 and D18S61, were amplified by PCR on the DNA from the YAC clones. The STS, STR and EST sequences used, are described from page 36 to 54. Positive YAC clones were assembled in a YAC contig map (Figure 14).

Three gaps remained in the YAC contig, of which one, between D18S876 and GCT3G01, was located in the refined candidate region. To close the gap between D18S876 and GCT3G01, 14 YAC clones (Table 3, on page 62) were further analysed. End fragments from YAC clones 766.f.12 (SV11R), 752.g.8 (SV31L), 942.c.3 (SV10R) were obtained and sequenced (see pages 55-61). Primers from these three sequences were selected, and DNA of each of the 14 YAC clones was amplified by PCR. As indicated in Table 3, overlaps were obtained between 7 YAC clones on the centromeric side, and two YAC clones on the telomeric site (717.d.3 and 907.e.1).

The final YAC contig is shown in Figure 14. In the figure, only the YAC clones which rendered unambiguous hits with the chromosome 18 STSs, STRs and ESTs are shown. In a few cases, weak positive signals were also obtained with some of the YAC clones, which

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likely represent false positive results. However, these signals did not influence the alignment of the YAC clones in the contig. Although, all YACs known to map in the region were tested as well as all available STSs/STRs, initially, the gap in the YAC contig was not closed. However, this was subsequently achieved by determining the end-sequences of the eight selected YACs (see below). The order of the markers provided by the YAC contig map is in complete agreement with the marker order provided by the WI map which integrates information from the genetic map, the radiation hybrid map and the STS YAC contig map (Hudson et al. 1995). Also, the YAC contig map confirms the order of the STR markers as suggested by the haplotype analysis in family MAD31. Moreover, the YAC contig map provides additional information on the relative order of the STR markers. For example, D18S55 is present in YAC 931_g_10 but not in 931_f_1 (Fig.14), separating D18S55 from its cluster [S55-S969-S1113-S483-S465] obtained by haplotype analysis in family MAD31. The centromeric location of D18S55 is defined by the STS/STR content of surrounding YACs (Fig. 14). If we combine the haplotype data and the YAC contig map the following order of STR markers is obtained: cen-[S51-S68-S346]-S55-[S969-S1113]-[S483-S465]-S876-S477-S979-S466-[S817-S61]-tel.

Out of the 25 YAC clones spanning the whole contig, seven YAC clones were selected in order to identify the minimal tiling path (Table 4). These 7 YAC clones cover the whole refined chromosome 18 region. Furthermore, YAC clones should preferably be non-chimeric, i.e. they should only contain fragments from human chromosome 18. In order to examine for the presence of chimerism, both ends of these YACs were subcloned and sequenced (pages 55 to 61). For each of

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the sequences, primers were obtained, and DNA from a
monochromosomal mapping panel was amplified by PCR
using these primers. As indicated on pages 55 to 61,
some of the YAC clones contained fragments from other
5 chromosomes, apart from human chromosome 18.

Three YAC clones were then selected
comprising the minimum tiling path (Table 5). These
three YAC clones were stable as determined by pulsed
field gel electrophoresis and their sizes correspond
10 well to the published sizes. These YAC clones were
transferred to other host yeast strains for
restriction mapping, and are the subject to further
subcloning.

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Description of the successfully analysed STSs, STRs and ESTs covering the refined candidate region.

Explanations:

- STS: Sequence Tagged Site
- STR: Simple Tandem Repeat
- EST: Expressed Sequence Tag

These markers are ordered from the centromere to the telomere. Only the markers that were effectively tested and that worked on the YACs are given.

List:

1. D18S60:

Database ID: AFM178XE3 (Also known as 178xe3, Z16781, D18S60)
Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs
Chromosome: Chr18

Primers:

Left = CCTGGCTCACCTGGCA
Right = TTGTAGCATCGTTGTAATGTTCC
Product Length = 157

Review complete sequence:

AGCTATCCTGGCTCACCTGGCAAAAATACAGTGTATACACACACACACAC
ACACACACACACACACACAGAGTGTNTTANTNATTCCAGCAAATAATATTA
CATATAAAAGATCTAATTGGTTCATCATGTAAATTTAGTAGGAACATTACA
ACGATGCTACAAGANTTTATCCAAAACCTGAGATTTCTTAGAATATCTGTT
AAAAGTAATTTTATTCAGTTAATAGAAATTCTATTGAAAACATCAAACCTTAT
AAAGCT

Genbank ID: Z16781

Description: H. sapiens (D18S60) DNA segment containing (CA) repeat;
clone

Search for GDB entry

2. WI-9222

Database ID: UTR-03540 (Also known as G06101, D18S1033, 9222,
X63657)

Source: WICGR: Primers derived from Genbank sequences

Chromosome: Chr18

Primers

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Left = GATCCCATAAAGCTACGAGGG

Right = GAGTCTAAAGACAAGAAAGCATTGC

Product Length = 99

Review complete sequence:

TCTTCTTACCCCTTGGGAAGAAGACTGTTTCCAAATAATTTGAACAGCTTG
CTGCTAAATGGGACCCAATTTTTGGCCTATAGACACTTATGTATTGTTTTT
GAATACGTCAGATTGGACCAAGTGTCTTCAGGAATGTGGCTGCAAGCAA
GGGGCTAGAAGTTCACCTCCTGACAGTATTATTAATACTATGCAAAATATG
GAATAGGAGACCATTTGATTTTCTAGGCTTTGTGGTAGAGAGGTGAAGG
TATGAGAATTAATAGCGTGTGAACAAAGTAAAGAACAGGATTCCAGAATG
ATCATTAAATTTGTTTCTATTTATTCTTTTTTGGCCCCCTAGAGATTAAGTC
CAGAAATGTACTTTCTGGCACATAAAGAAATCTTGAGGACTTTGTTTAAAC
CTTCCATAAAAAACAATTTTCGGTTCCTCGGGTNNNNNNNNNNNNNNNNNT
NNNT
TCTTCTTTGTGTATTTTATTCAAGATGAGTTGGACCCATTGCCAGTGAGT
CTGAATGTCACTGACAGCCCTGTGTTGTGCTCAGGACTCACTCTGCTGC
TGGTGGAAACTCATGGCTTCTCTCTCTCTTTGATCCCATAAAGCTACGAG
GGGGACGGGAGAGGGCAGTGCAATGGGAAGTAAAGAGATATTTTCCAG
TAGGAAAAGCAATGCTTTCTTGTCTTTAGACTCAAATGCTTAGGGAACGT
TTCATTTCTCATTTCATGGGGAAAGGCAGCCTCCTTAAATGTTTTCTGAAG
AGCGGTAAAATCTAGAAGCTTAAGAATTTACAGTTCTTCAATAACCATGA
TGACCTGAAGTTCACCTATCCCATTTAGCATCTACTTGTTTTTCCCATCT
CTTCCTTTCCAATTTTGCTTATACTGCTGTAATATTTTGTNNNNNNNNNN
NNNNNNNNNNNNNNNGACCAGCTAAAATTTTCGACTTGACTTTTTAACTT
AACTCATGAATTAATTAAGCAAATGAAAAAATTAAGAGTGTGACTTTTT
CTCGGAGCATATATGTAGCTTTTAGGAAAGGCTGATGATGGTATAAAGTT
TGCTCATTAAGAAAAAAGACAAGGCTGATTTTGAAGAGAGTTGCTTTTG
AAATAAATGATCA

Genbank ID: X63657

Description: H.sapiens fvt1 mRNA

Search for GDB entry

3. WI-7336:

Database ID: UTR-04664 (Also known as PI5, G00-679-135, G06527, 7336, U04313)

Source: WICGR: Primers derived from Genbank sequences

Chromosome: Chr18

Primers:

Left = AGACATTCTCGCTTCCCTGA

Right = AATTTTGACCCCTTATGGGC

Product Length = 332

Review complete sequence:

TAAGTGGCATAGCCCATGTAAAGTCCTCCCTGACTTTTCTGTGGATGCCG
ATTTCTGTAAACTCTGCATCCAGAGATTCATTTTCTAGATACAATAAATTG
CTAATGTTGCTGGATCAGGAAGCCGCCAGTACTTGTCATATGTAGCCTTC

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ACACAGATAGACNNNNNNNNNNNCCAATTCTATCTTTGTTTCCTTTTT
 CCCATAAGACAATGACATACGCTTTTAATGAAAAGGAATCACGTTAGAGG
 AAAAATATTTATTCATTATTTGTCAAATTGTCCGGGGTAGTTGGCAGAAAT
 ACAGTCTTCCACAAAGAAAATTCCTATAAGGAAGATTTGGAAGCTCTTCT
 TCCCAGCACTATGCTTTCTTTCTTTGGGATAGAGAATGTTCCAGACATTC
TCCGCTTCCCTGAAAGACTGAAGAAAGTGTAGTGCATGGGACCCACGAAA
 CTGCCCTGGCTCCAGTGAAACTTTGGGCACATGCTCAGGCTACTATAGGT
 CCAGAAGTCCTTATGTTAAGCCCTGGCAGGCAGGTGTTTATTAAAATTCT
 GAATTTTGGGGATTTTCAAAAGATAATATTTTACATACACTGTATGTTATA
 GAACTTCATGGATCAGATCTGGGGCAGCAACCTATAAATCAACACCTTAA
 TATGCTGCAACAAAATGTAGAATATTCAGACAAAATGGATACATAAAGACT
 AAGTAGCCCATAGGGGGTCAAAATTTGCTGCCAAATGCGTATGCCACCA
 ACTTACAAAAACACTTCGTTTCGCAGAGCTTTTCAGATTGTGGAATGTTGG
 ATAAGGAATTATAGACCTCTAGTAGCTGAAATGCAAGACCCCAAGAGGAA
 GTTCAGATCTTAATATAAATTCACTTTCATTTTGTATAGCTGTCCCATCTG
 GTCATGTGGTTGGCACTAGACTGGTGGCAGGGGCTTCTAGCTGACTCG
 CACAGGGATTCTCACAATAGCCGATATCAGAATTTGTGTTGAAGGAACTT
 GTCTCTTCATCTAATATGATAGCGGGAAAAGGAGAGGAACTACTGCCTT
 TAGAAAATATAAGTAAAGTGATTAAAGTGCTCACGTTACCTTGACACATAG
 TTTTTCAGTCTATGGGTTTAGTTACTTTAGATGGCAAGCATGTAACCTATA
 TTAATAGTAATTTGTAAAGTTGGGTGGATAAGCTATCCCTGTTGCCGGTT
 CATGGATTACTTCTCTATAAAAAATATATTTACCAAAAAATTTTGTGACA
 TTCTTCTCCCATCTCTTCTTGACATGCATTGTAATAGGTTCTTCTTGT
 TCTGAGATTCAATATTGAATTTCTCCTATGCTATTGACAATAAAATATTATT
 GAACTACC

Genbank ID: G06527

Description: WICGR: Random genome wide STSs

4. WI-8145:

Database ID: EST102441 (Also known as D18S1234, G00-677-827, G06845, 8145, T49159)

Source: WICGR: STSs derived from dbEST sequences

Chromosome: Chr18

Primers:

Left = GAAATGCACATAACATATATTTGCC

Right = TGCTCACTGCCTATTTAATGTAGC

Product Length = 184

Review complete sequence:

GTTGTTTGGANGCAGGTTTATTTATTATATACTTGCAATTGAATATAAGAT
 ACAGACATATATATGTGTTATGTATTTCTAGAAATGCACATAACATATATTT
GCCTATTGTTTAATGTTTTTCCAGANATTTATTACAGAAGGGCATGGAG
 GGATACCTACTTATTCTTCATTATGAGAACAATTAAAGGCATTTATTAGAT
 AGGAAATTAACAGANCATCTGCTTCTATAACTTTATTAGCTACATTAAATA
GGCAGTGAGCANTAATTTAAAANCTCACCATTATATAAANTANTAAATACC
 AAAGTAAAAG

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_____ : left and right primer

PCR Conditions

Genbank ID: T49159

Description: yb09e07.s1 Homo sapiens cDNA clone 70692 3' similar to gb:J02685

UniGene Cluster Description: Human mRNA for Arg-Serpin (plasminogen activator-inhibitor 2, PAI-2) Search for GDB entry

5. WI-7061:

Database ID: UTR-02902 (Also known as PAI2, G00-678-979, G06377, 7061, M18082)

Source: WICGR: Primers derived from Genbank sequences

Chromosome: Chr18

Primers:

Left = TGCTCTTCTGAACAACTTCTGC

Right = ATAGAAGGGCATGGAGGGAT

Product Length = 338

Review complete sequence:

AACTAAGCGTGCTGCTTCTGCAAAAAGATTTTTGTAGATGAGCTGTGTGCC
TCAGAATTGCTATTTCAAATTGCCAAAATTTAGAGATGTTTTCTACATAT
TTCTGCTCTTCTGAACAACTTCTGCTACCCACTAAATAAAAACACAGAAAT
AATTAGACAATTGTCTATTATAACATGACAACCCTATTAATCATTTGGTCT
TCTAAAATGGGATCATGCCCATTTAGATTTTCCTTACTATCAGTTTATTTT
TATAACATTAACTTTTACTTTGTTATTTATTATTTTATATAATGGTGAGTTTT
AAATTATTGCTCACTGCCTATTTAATGTAGCTAATAAAGTTATAGAAGCAG
ATGATCTGTTAATTTCTATCTAATAAATGCCTTTAATTGTTCTCATAATGA
AGAATAAGTAGGTATCCCTCCATGCCCTTCTATAATAAATATCTGGAAAAA
ACATTAAACAATAGGCAAATATATGTTATGTGCATTTCTAGAAATACATAA
CACATATATATGTCTGTATCTTATATTCAATTGCAAGTATATAATAAATAAA
CCTGCTTCCAAACAACNNNNNNNNNNNNNNNGGAATTC

PCR Conditions

Genbank ID: G06377

Description: WICGR Random genome wide STSs

6. D18S68

Database ID: AFM243YB9 (Also known as 248yb9, Z17122, D18S68)

Source: J Weissenbach, Genethon. genetically mapped polymorphic STSs

Chromosome Chr18

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Primers:

Left = ATGGGAGACGTAATACACCC

Right = ATGCTGCTGGTCTGAGG

Product Length = 285

Review complete sequence:

AAAGAGTTGGGGTTGTGAATTCACACACAGTCAACTATTGGCTATGGG
CTTACCATGGGAGACGTAATACACCCGGNACTTCCAACCTCACATACCAG
AGACATGGCTCTAGCACCCCAATGGAAATATGCTGAATGTTGCAGGTGCA
AGACAGCAACAAAGCAGACAGAGGCACATAGACAAGGCACCAACAGTGT
CCACTATACCCTGACAGTGTGGAAAGTTGTAGATAGGATGAAGAGAAAG
AATACA
CGGTAGANACTTACTACNCAAAGTGTGANCCCTCAGACCAGCAGCATCTG
GCNAAATGGTGATCTATCACCTTCCAG

Genbank ID: Z17122

Description: H. sapiens (D18S68) DNA segment containing (CA) repeat;
clone**7. WI-3170:**

Database ID: MR3726 (Also known as D18S1037, G04207, HALd22f2, 3170)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = TGTGCTACTGATTAAGGTAAAGGC

Right = TGCTTCTTCAATTTGTAGAGTTGG

Product Length = 156

Review complete sequence

CTGAGACAAGGCAGGCCAAACAACCTCTAAAAATCTACAATTGGTGATTGG
TGTGCTACTGATTAAGGTAAAGGCACAGAATTATACATCCAGGTTNCTAT
TACTTATGGCAGACTCAGGACCCAGGTTNAGAGACCACTGGCCTTAAGA
AAAAAAATGGGGTTCCTGATTTCTGGATAATAATCCAACCTCTACAAATTGA
AGAAGCAACATACCCTCTTTGTTA

Genbank ID: G04207

Description: WICGR: Random genome wide STSs

8 WI-5654:Database ID: MR10908 (Also known as D18S1259, G00-678-695, G05278,
5654)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

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Primers:

Left = CTTAATGAAAACAATGCCAGAGC

Right = TGCAAAATGTGGAATAATCTGG

Product Length = 149

Review complete sequence:

CTACAAAATGCATGTGGCTTTGGCTTTGAAATAGTACACCCTATCAAAGA
CTAAATTTTCTTAATGAAAACAATGCCAGAGCTTTTTTCATGATATTTGTT
TTTAGAGATGGGGAACAATCTGGACGTTGTTTCCTTATCTGGGTGGTAAT
CGAGGCTTAGCAATTTCCACAGCGTTACACAAATCCAGATTATTCCACA
TTTTGCAAATA

Genbank ID: G05278

Description: WICGR: Random genome wide STSs

9. D18S55:Database ID: AFM122XC1 (Also known as 122xc1, Z16621, D18S55,
GC378-D18S55)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = GGGAAGTCAAATGCAAAATC

Right = AGCTTCTGAGTAATCTTATGCTGTG

Product Length = 143

Review complete sequence:

AGCTGAACATGCCTTTTCATGGAGCAGTTTCNAAATACACTTTTGGTACA
ATCTGCAGGTGGATATTTGGAGCTCAGGAGTTTGAGACCAGCCTGGGCA
ACATGGTGAAATCCCGTCTCTACTAAAATACAAAAAATTAGCCAGGTGTG
GCGGCATGTGCCTGTAGNCCCAGGATGGATTGAGTGGGTGAGATATGG
AATAAGTGGTGGGAAGTCAAATGCAAAATCAATTCAGTTTGTCAATATTG
ATTCTCTATTCTAGCCTGGCGTGGTTTTTCCTCGTCACACACACACACAC
ACACACACACACACACACACACACACACACACAGCATAAGATTACTCAGA
AGCT

Genbank ID: Z16621

Description: H. sapiens (D18S55) DNA segment containing (CA) repeat;
clone10. D18S969:Database ID: GATA-P18099 (Also known as G08003, CHLC.GATA69F01,
CHLC.GATA69F01.P18099)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome Chr18

Primers:

Left = AACAAGTGTGTATGGGGGTG

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Right = CATATTCACCCAGTTTGTTC

Product Length = 365

Review complete sequence:

CAGGGAAATGCAAATCAAACCCACAATGAGTTATCTCCTCATACCTTTAAT
GATGGCTAATATTAAACAAGAGATAACAAGTGTGTATGGGGGTGTGGAG
AAAAGAGAATGTNCGAACACTCTTGGTTGAAATATAAGTTGGTAGANCCA
TTATGCAAAACAGTATGAATCTTTATCAGTATAANATTAGGACCTNGCATA
TGATCNCAGCAATCNCACNTCTGNGNGATCNCACNCNCTATCTCTCTAT
ATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCT
ATCTGTCTGTCTATCATCTATCTATCTTCTATCTATCTATCTATCTTTCTAT
CTATCTATCTGTCTATCTATNCCGGAATATTTTTCAGCCATNNAATAAGG
AAGTCCTGCTATTTGCAACAAACTGGGTGAATATGGAGAACGTTATGCTA
AATGCAATATGCTAAAGACAGACACAGAAAGACAAGTATGACCTCACTTA
TATGTGGAAACTGAAAAAGCCATACTCATTACAGCAAAGAGTAGAATGTT
GGTTACCAGGGGCAAAGAGGGTAGAAATGAGGGGAGTGAGAAAATGTC
AATCAAAGTGTAAGAATGTTATAACATAAATAAATTCATAGAG

Genbank ID: G08003

Description: human STS CHLC.GATA69F01.P18099 clone GATA69F01.

11. D18S1113:

Database ID: AFM200VG9 (Also known as D18S1113, 200vg9, w2403)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = GTTGACTCAAGTCCAAACCTG

Right = CAAAGACATTGTAGACGTTCTCTG

Product Length = 207

Review complete sequence:

AGCTGCATATAAACTATTCCATTTTCACATTTTGAAGACATTTGTAGCCA
TGATACTTTGCTGTTGTCTGTGGGCCACCTCTTTTTGAAGTGTGTAGTTA
ACTGTGCTCCTGTAATCTGTTGTCTGTTGACTCAAGTCCAAACCTGTTCT
GCGTGGCATGTTTCTNCAACTTGATGTGATGCTATTTATCACTTTCTTTGA
AGTTAAGTCTCTATGTCTTTGTATTCTTTCTGTGTACCCAGGGATATGTTT
GTGCATGCACACGCATAAACACACACACACACACACACACACAGAGA
CAGAGACAGAGAACGCTCTACAATGTCTTTGTGAG

12. D18S868:

Database ID: GATA-D18S868 (Also known as G09150, CHLC GATA3E12,
CHLC.GATA3E12.496 CHLC.496, D18S868)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

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Primers:

Left = AGCCAATACCTTGTAGTAAATATCC

Right = GATTCTCCAGACAAATAATCCC

Product Length = 189

Review complete sequence:

GAGTGAGCCAATACCTTGTAGTAAATATCCATCTATCTTTGATGTATCTAT
GTATCTATCTTTGTATCTATATGTCTATGTATCTATGTATGTATGTATCTAT
CTATCATCTATCTATCTATCATCTATCTATCTATCTATCTATCTATCTATCT
ATCTATCTATATCCNTTTGGGATTATTTGTCTGGAGAATCCTGATTAAAT
AGTCTGCTAACTTTTATCTGTATCTCCTATGTGTATGCTTCTCCTTCTTCC
TGTCTCTCTCTTCTTTGTCCCTCATTTAANCTCCTTTCCTGGGNATATTG
GNAATTTTGATTGGANTCTGGACANTGTAGGAGTAAAAATTT

Genbank ID: G09150

Description: human STS CHLC.GATA3E12.P6553 clone GATA3E12.

13. WI-9959:

Database ID: MR12816 (Also known as D18S1251, G00-678-524, G05488, 9959)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = TGCCAACAGCAGTCAAGC

Right = AGCACCTGCAGCAGTAATAGC

Product Length = 110

Review complete sequence:

ctgttttatttgaaaaaaaaaatctgtctccaagaagaaaagttcattcACCTGTTGCCAACAGC
AGTCAAGCGGACATGTTTAAAATTTTTTAAAAAAGTATTTTTTTTCCAAC
GGNGTTTAATAGCCTCATTTTGGCTTTTGCTATTACTGCTGCAGGTGCTT
TNATTTTTTTCCTCTGCATTATAATTAC

Genbank ID: G05488

Description: WICGR: Random genome wide STSs

Search for GDB entry

14. D18S537:

Database ID: CHLC GATA2E06.13 (Also known as CHLC.13, GATA2E06, D18S537, GATA-D18S537)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = TCCATCTATCTTTGATGTATCTATG

Right = AGTTAGCAGACTATGTTAATCAGGA

Product Length = 131

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Review complete sequence:

AAAGCTGAGTGAGCCAATACCTTGTAGTAAATATCCATCTATCTTTGATGT
ATCTATGTATCTATCTTTGTATCTATATGTCTATGTATCTATGTATGTATGT
ATCTATCTATCATCTATCTATCTATCATCTATCTATCTATCTATCTATCTAT
CTATCTATCTATCTATATCCNTTNGGTATTATTNGTCTGGNGAATCCTGAT
TAACATAGTCTGCTAACTTNTATCTGTATCTNCTATGTGTATGCTTCTNCT
TCTTCCTGTCTCTCTCTCTGCTTTGTCTCAATTNAAATCTCC

Genbank ID: G07990

Description: human STS CHLC.GATA2E06.P6006 clone GATA2E06.

Search for GDB entry

15. D18S483:

Database ID: AFM324WC9 (Also known as 324wc9, Z24399, D18S483)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = TTCTGCACAATTTCAATAGATTC

Right = GAACTGAGCAAACGAGTATGA

Product Length = 214

Review complete sequence:

AGCTCTGCTGGAAGAGCAGGGCTGTTTTCTGCACAATTTCAATAGATTC
CCTACCCTGGGTTTTTTCAGTAGATAGATAGATAGATGATAGATAGGTAGA
TAGATAGATAGATAGATAGATAGATAGATAGATAGATGATAGATAGATTTT
ATATATAGTATATAAAATCTACACACACACACACACACACACACACATA
TTTGCCTTTTCTTGACTATCATACTCGTTTGCTCAGTTCTTTTTTTTTTAA
ATTTTTGTTTGTAATCCAAATGCTT

Genbank ID: Z24399

Description: H. sapiens (D18S483) DNA segment containing (CA) repeat;
clone

Search for GDB entry

16. D18S465:

Database ID: AFM260YH1 (Also known as 260yh1, Z23850, D18S465)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = ATATCCCCCTATGGAAGTACAG

Right = AAAGTTAATTTTCAGGCACTCT

Product Length = 232

Review complete sequence:

AGCTCTGTCCCTCTAGAGAACGCTGACTAATATATTTCCCCTATGGAAGTA
CAGATGGTTTTNTAAAATAAATTTATCTGATTGTGATGAGATAATCATCA

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TTTTATGTTTCAGTGTCTTCTAAATTTTATTGTTATTGTTTTATACTCT
AAATGGTTTTTAAATATGCACATATGTGCATATTTACACACACACACACA
CACACACACACTCTCTTTATTTAGAAGCATTATAGATAGAGTGCCTGAAAA
TTAACTTTTAACCNAAGAAAAGACAATAAGGAACAATAGGGAAGTTATCC
TTTGCTAAGGGTATGGAAAATATTCACATATTATTTATAACANGTTAAACC
AAGTCATGCTTGANTATAATAGCT

Genbank ID: Z23850

Description: H. sapiens (D18S465) DNA segment containing (CA) repeat;
clone

Search for GDB entry

17. D18S968:

Database ID: GATA-P34272 (Also known as G10262, CHLC.GATA117C05,
CHLC.GATA117C05.P34272)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = GAAATTAACCAGACACTCCTAACC

Right = CTTAGAATTGCCTTTGCTGC

Product Length = 147

Review complete sequence:

GAATAAAAATATGAGGTATTAGAAATTTACAGATAGGAAGAAATTAACCAG
ACACTCCTAACCACCGATNAGTTTAAAGAGGAGATAGATAGATAGATGAT
AGATAGATAGATAGATAGATAGATACCACTGAAAATGCAANCACAAATTA
GCAGATTATATGTGATGCAGCAAAGGCAATTCTAAGTAGATTCTAACTGC
TACATTGATAGCAGTACCCACTGACATTACCGGAAAGGATGGTATCCATA
ACCACCTACCTATATACCTCCGCAGCTGGANATTAGGNTTAAGCTTCTTN
GGGCNCCTGGCGGCCCCCNNTTGTGGTCCCCGGTNGGNCCCCGNTTNN
GNNTNGCTNNGNTTNCNTTGGNGNCCCCCNNTNGGTTTNNGGNNNNNT
NNNNNTNGNNNNNTTNCNNNNNNNNNTNTNNNNCNNNNNNNNNTNNN
NNNNNNNNNGGNNNNNGGNN

Genbank ID: G10262

Description: human STS CHLC.GATA117C05.P34272 clone GATA117C05.

18. GATA-P6051:

Database ID: GATA-P6051 (Also known as CHLC.GATA3E08,
CHLC.GATA3E08.P6051)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = GCAACAACCCTAATGAGTATACG

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Right = GAGTCTCACCAGGGCTTACA

Product Length = 149

Review complete sequence:

AAAGCTGTCTCCTTTTGTAAAGTGTGCTCAGAGGAATCTTTTTCAGTAAAT
AAAGTCTGCACCCAGACATCTCACTTTGTATACCACGGAGAATTTACCAT
GACTCTTCTCAGTGATAAACGTCAATATAGAATAATCAGGAGAAAAAGAG
AAATCCAGTAAAGAAATAAGTCTGTAGAAAGCAACAACCCTAATGAGTAT
ACGATATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATG
NATCTATCTATCTAACATTATATAAAATATATATTTCTCTGTATTGGGG
CCCTGTGTGTAAGCCCTGGTGAGACTCAAAAATTTGANTATTCCTNTTTN
T

Genbank ID: G09104

Description: human STS CHLC.GATA3E08.P6051 clone GATA3E08.

19. D18S875:

Database ID: GATA-D18S875 (Also known as G08001, CHLC.GATA52H04, D18S875)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers.

Left = TCCTCTCATCTCGGATATGG

Right = AAGGCTTTCAGACTTACACTGG

Product Length = 394

Review complete sequence:

TTATTTATTCACTCATTCAATAAATATTTATGAATTTCTTTAATGGCNANG
AAAGTATGTTTGGTACTGAATATGGTGAGCAAGATTTTCTCTCATCTCG
GATATGGAAAGATCTTGGAAATCATTATACNTCATACTTACAATANGAAAG
AAGCTGAGCAATTTGAAAATCAACAATTTCTTTGTACNTGTCAGAAAAGT
GAAGATATATTAATCAGGGTTCTTCAGAGAAACATAACCAATAGGNCACA
GNTCTATATGNCCNCNTTTATCTATCTATCTATCTATCTATCNCATCTAT
CNCANACCNGGNGAANTNATNTTTGNGAGATTNATGCAAGNCTGAGAAA
NACCNAAGAANCTGCTCCCTGTNAACTNGAGATNCAAGAANCTGAANA
GTATAGNTCCAGTCCNAAGTCTANAGACCTTAGAATTAGGAAAAGTATA
CTATAAATACCAGTGTAAGTCTGAAAGCCTTAAANACCANATAGTGCCAT
TGAAAGGGCAGAAGACTGATGTCCAGTTCAAGCAGGCAAAGTTAGAGA
AGCCTTATTTTCTGCAACATTGTTCTATTTCAGACCCTTNANANGATTGACN
ATGTCCACCCA

Genbank ID: G08001

Description: human STS CHLC.GATA52H04 P16177 clone GATA52H04.

Search for GDB entry

20. WI-2620

Database ID: MR1436 (Also known as G03602, D18S890, HHAa12h3, 2620)

Source: WICGR Random genome wide STSs

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Chromosome: Chr18

Primers:

Left = TCTCCAAGCTATTGATTGGATAA

Right = TTAAGAGCCAATTTATATAAAAGCAGC

Product Length = 177

Review complete sequence:

CCCCTTTTGCCAACGCCATGCTTCACGTAGGGAGCCTGACATGCAGAAA
ACTCTCCAAGCTATTGATTGGATAAAGAGCCAGAGCTGACTGAATTCCAT
TCTTCTTGAGCCTCTCATTCTGTGTTTCTCGAATTTTACCAAAGCATCTT
GACACACAAATATCTGACTCAAGGAAAAGGAAAAACAACCTGCTTTTCTC
CAGCTGCTTTTATATAAATTGGCTCTTAACTTTCTAAGTTTATTATGGAT
A

Genbank ID: G03602

Description: WICGR: Random genome wide STSs

Search for GDB entry

21. WI-4211:

Database ID: MR6638 (Also known as G03617, D18S980, 4211)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = ATGCTTCAGGATGACGTAATACA

Right = AAATTCTCGCTGATTGGAGG

Product Length = 113

Review complete sequence:

CTAGTACCATAATCCCTTTTGGGAATAAACCATCCCACCTTTAGTCAGANC
AGATGCTTCAGGATGACGTAATACATAATAAGCCTACTCAGTTCTACTCT
GGCTTTGTATGTCTTCAAAGTGATATTTTTTAAGTATTACTTGTCCCTCC
AATCAGCGAGAATT

Genbank ID: G03617

Description: WICGR: Random genome wide STSs

Search for GDB entry

22. D18S876:

Database ID: GATA-D18S876 (Also known as G09963, CHLC.GATA61E10, D18S876)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = TCAAACCTTATACTGCAGAGAACG

Right = ATGGTAAACCCTCCCCATTA

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Product Length = 171

Review complete sequence:

AAGACTGCAATTACATTTGCATCAAACCTTATAACTGCAGAGAACGTTGCC
CACTATTTTATACCACACAACAGTATTCTTAGCCAGATTACATCTATCTAT
CTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTATCTAGC
TAGCTATCTATCTATAGAATAATGGGGAGGGTTTACCATGTTTGGGTGA
ACCCAAACATTTTATGGNCAAGGGNTTGGAAAATTACCCTTATCTACAAC
TNTTNAACCTGTTTTGGTAGGNGTGNTAATTCNTGGGNTTGAANAAC
TTTGNAATTTCTCCTCNTTGTNTNATTNNNNATTNNTNNNCATTATTNTGG
GGTNTTCNGGGTGGAGGGCTNANTTTGGCCNCCCGGGTCCNNGGNGC
NAGTNGGNNNGGNTNNTNNGGGTTTCTTGGGAANCNTNCCNCCTNCNG
GGGNTTCANGGGNTTTTTNTTTNNTTG

Genbank ID: G09963

Description: human STS CHLC.GATA61E10.P17745 clone GATA61E10.

Search for GDB entry

23. GCT3G01:Database ID: GCT-P10825 (Also known as G09484, CHLC.GCT3G01,
CHLC.GCT3G01.P10825)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = CTTTGCAATCTTAGTTAATTGGC

Right = GAACTATGATATGGAGTAACAGCG

Product Length = 128

Review complete sequence:

AGATGTTTAACTTTGCAATCTTAGTTAATTGGCAGAAATGAAATTTAGTTT
CCACAACTTTTATTGATATTAACACACCACCACCATCAGCAGCAGCAGC
AGCAGCAGCAGCATCGCTGTTACTCCATATCATAGTTTCAGAGCATTAAA
GNGGTCAAAATATACAACTAGGCTGACACCNGNATAAGGTTTAAATTTAA
ACCNNGGCTCTNCCCTCTAAGGNGGNTTTTTTTTTCTTGNCNTGGCTTCT
TTTTCCNTTTGCTTTTGTAATAATCAAGGNATTTTGGGTTNTTCNTGGN
ANTTNNCANNANTNNTNNTTNNNCNCNCCCCCCTTTGNGGCGGGGGTC
CCCNNTTGTCCCGGGGTTGNGTGCAGTAGGGGGGTCNCGGGTNNNG
NAAGTTTNGGGGCCCT

Genbank ID: G09484

Description: human STS CHLC GCT3G01.P10825 clone GCT3G01.

24. WI-528:

Database ID: MH232 (Also known as G03589, 528, D18S828)

Source: WICGR: Random genome wide STSs

Chromosome: Chr13

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Primers:

Left = TTCTGCCTTTCCTGACTGTC

Right = TGTTTCCCATGTCTTGATGA

Product Length = 211

Review complete sequence:

CTACTAAGCAAATTCTGCTCAGCCTTCTGCCTTTCCTGACTGTCCTTGTTG
GCCCTTCCCACCTTTAAGGATGCCTGTTTAAGTAGCCACCTCTAATTAGGA
ATCTTCCCTTGTTCTTCTCAGGAGGCTTAGACACTGTCAGTTTCCTGAA
GACAGAAAATAAGCCTGCATTATCCTAGTAGTGGATTCAAACTAATTGT
GTCCTGAGTCTTTCAATCATCAAGACATGGGAAACACTCAACAG

Genbank ID: G03589

Description: WICGR: Random genome wide STSs

Search for GDB entry

25. WI-1783:

Database ID: MR432 (Also known as G03587, _shu_31.Seq, 1783,
D18S824)

Source: WICGR: Random genome wide STSs

Chromosome: Chr18

Primers:

Left = CCAGTAATTAGACATTGACAGGTTT

Right = TTTTACTAGACAGGCTTGATAAACAA

Product Length = 305

Review complete sequence:

CCAGTAATTAGACATTGACAGGTTCCATACTAGTAATGTAGGGAATAGGG
CTGCTGCTTTTTTGGGTTTTCTTGAGTATACTTTGTGCTGCATAAATATGG
CAATGGATAGTAAATAATTTGTATGCAGACCTTTAGTGTGCGATTAACTGT
GAATAAGGGAACAACAATCAAGGACAAAAATCAAAGACTAATTCTCTAT
ACATTTTGAGCTTTTGTAAAAAAGTAAGATTAGCTGAATATATCTGAAAAA
TTTCTAATCTCCTTTACAATTTTTTAAATTGTTTATCAAGCCTGTCTAGTAA
AATAATTCAGTTTCGGAATGTGG

Genbank ID: G03587

Description: WICGR: Random genome wide STSs

Search for GDB entry

26. D18S477:

Database ID: AFM301XF5 (Also known as 301xf5, Z24212, D18S477)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = GGACATCCTTGATTTGCTCATAA

Right = GATTGACTGAAAACAGGCACAT

- 50 -

Product Length = 243

Review complete sequence:

GGACATCCTTGATTGGCTCATAATACACTCATTCTTTACCAATTGAGTGT
GCACATATTTCTCTGATTGGAAAGAACTACAGAGGAGGTTTTACNTTTTA
CTTTCCAGTTTGCTATTAAAGAGAGAAAACTAACAGAGNGAAATCAAGCA
ACTCAAAACAACCTTACACACACACACACACACACTCACAAAGATA
TTTTGTTACCATATGTATTGATGTGCCTGTTTTCAGTCAATCCACAGGAA
GGGCTAAGGAGAGTGACATCTGGGCTACATTAAAAGGACAGTCACATTG
CTCAAAGNACTCAAGTTTAGCCCCGAGTACAGTAGCT

Genbank ID: Z24212

Description: H. sapiens (D18S477) DNA segment containing (CA) repeat;
clone

Search for GDB entry

27. D18S979:

Database ID: GATA-P28080 (Also known as G08015, CHLC.GATA92C08, CHLC.GATA92C08.P28080)

Source: CHLC: genetically mapped polymorphic tetranucleotide repeats

Chromosome: Chr18

Primers:

Left = AGCTTGCAGATAGCCTGCTA

Right = TACGGTAGGTAGGTAGATAGATTCG

Product Length = 155

Review complete sequence:

[illegible]

Genbank ID: G08015

Description: human STS CHLC.GATA92C08.P28080 clone GATA92C08.

28. WI-9340

Database ID: UTR-05134 (Also known as G06102, D18S1034, 9340, X60221)

Source: WICGR: Primers derived from Genbank sequences

Chromosome: Chr18

Primers

Left = TGAGAGAACGAAATCTCTATCGG

Right = AGGCAGCAAGTTTTTATAAAGGC

Product Length = 115.

Review complete sequence:

- 51 -

ATGTATCTATCCCAATTGAGTCAGCTAGAAACAGTTGACTGACTAAATGG
AAACTAGTCTATTTGACAAAGTCTTTCTGTGTTGGTGTCTACTGAAGTTAT
AGTTTACCCTTCCTAAAAATGAAAAGTTTGTTTCATATAGTGAGAGAACGA
AATCTCTATCGGCCAGTCAGATGTTTCTCATCCTTCTTGCTCTGCCTTTG
AGTTGTTCCGTGATCATTCTGAATAAGCATTTCCTTTATAAAAACTTGCT
GCCTGACTAAAGATTAACAGGTTATAGTTTAAATTTGTAATTAATTCTACC
ATCTTGCAATAAAGTGACAATTGAATG

Genbank ID: G06102

Description: WICGR: Random genome wide STSs

Search for GDB entry

29. D18S466:

Database ID: AFM094YE5 (Also known as 094ye5, Z23354, D18S466)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = ACACTGTAGCAGAGGCTTGACC

Right = AGGCCAAGTTATGTGCCACC

Product Length = 214

Review complete sequence:

aatgacactttaaggaggtaacactgtaqcagagqcttgaccaccaccagttctcactagcactgagg
atgctctattgggtgggttaccacacacgcacatagacatgcacacacacagacacacacacacac
acacacacacacaccagatatgacattccaaaccatcaatatgctatgcaatactgcattaacagggtcatg
cctgtggtggcacataactggcctagaaaatactggggacgtctgcattccctttattatcgaattgacttact
tggcttctgagttttctcagaagtaatacttcaatacctcttccattctgccttgancattgttggggtagcaag
tatagct

Genbank ID: Z23354

Description: H. sapiens (D18S466) DNA segment containing (CA) repeat;
clone

Search for GDB entry

30. D18S1092:

Database ID: AFMA112WE9 (Also known as D18S1092, w5374, a112we9)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = CTCTCAAAGTAAGAGCGATGTTGTA

Right = CCGAAGTAGAAAATCTTGGCA

Product Length = 163

Review complete sequence:

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agctctcaaaqtaaqagcgaatgttaactgactgagttgtttgtgaantttgntttggagtcagtggagcat
gttattagatgtaaatttaaacacacacacacacacacacacacacacacacagagaagtaagtqccaag
attttctacttcggcgccctatatattctatactgattttctgtatttcccagacttgaatatagattgtcttctgntttat
calagacaatctcataataanttaggcataataaggtaatgaggntttctgggcttctttcatcatccctgca
atttgagtctcnittatagnigaantcttctctgtaataacncttctgttttagct

Search for GDB entry

31. D18S61:

Database ID: AFM193YF8 (Also known as 193yf8, Z16834, D18S61)

Source: J Weissenbach, Genethon: genetically mapped polymorphic STSs

Chromosome: Chr18

Primers:

Left = ATTTCTAAGAGGACTCCCAAAC

Right = ATATTTTGAACTCAGGAGCAT

Product Length = 174

Review complete sequence:

CGTCTTACCAAACCAACATAATATAGCAATGGNAACCAAAAATTTCTAAGA
GGAATCCCAAACCTACATTCTTCTNCCTGAATTAAATACAGGCATTCAANA
NAAACANACACACACACACACACACACACACACACACACACACACGCACA
CCCTTCAAATCNTAGCATAAATTCNCTTATATAAACATAAACCATGCTCCT
GAGTTTCAAATATTGGGTGGTTCGAAGTTCGAAGCAACAAATTTCCAGT
TAGTGTCTATTANTTGTTGGACAGCT

Genbank ID: Z16834

Description: H. sapiens (D18S61) DNA segment containing (CA) repeat;
clone

Search for GDB entry

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Markers (STRs) used in refining the candidate region.

Below the markers are shown that were used in family MAD31 to refine the candidate region. Most of these markers are already described above and will therefore only be mentioned to by their name. For the additional markers, the information is given here.

Data was already shown for: D18S68, D18S55, D18S969, D18S1113, D18S483, D18S465, D18S876, D18S477, D18S979, D18S466 and D18S61.

New data:

1. D18S51:

Other names: UT574, (D18S379)

Primer sequences:

UT574a GAGCCATGTTTCATGCCACTG

UT574b CAAACCCGACTACCAGCAAC

DNA-sequence:

AATTGAGCNCAGGAGTTTAAGACCAGCCTGGGTAACACAGTGAGACCCC
TGTCTCTACAAAAAATACAAAAATNAGTTGGGCATGGTGGCACGTGCCT
GTAGTCTCAGCTACTTGCAGGGCTGAGGCAGGAGGAGTTCTTGAGCCCA
GAAGGTTAAGGCTGCAGTGAGCCATGTTTCATGCCACTGCACCTTCACTCT
GAGTGACAAATTGAGACCTTGTCTCAGAAAGAAAGAAAGAAAGAAAGAAA
GAAAGAAAGAAAGAANGAAAGAAAGAAAGTAAGAAAAAGAGAGGGAAAG
AAAGAGAAANAGNAAAANAATAGTAGCAACTGTTATTGTAAGACATCTCC
ACACACCAGAGAAGTTAATTTTAATTTTAACATGTTAAGAACAGAGAGAAG
CCAACATGTCCACCTTAGGCTGACGGTTTGTTTATTTGTGTTGTTGCTGG
TAGTCGGGTTTGTTATTTTAAAGTAGCTTATCCAATACTTCATTAACAAT
TTCAGTAAGTTATTTTCATCTTCAACATAAATACGNACAAGGATTTCTTCT
GGTCAAGACCAAACTAATATTAGTCCATAGTAGGAGCTAATACTATCACA
TTTACTAAGTATTCTATTTGCAATTTGACTGTAGCCCATAGCCTTTTGTGCG
GCTAAAGTGAGCTTAATGCTGATCGACTCTAGAG

GENBANK ID: L18333

2. D18S346

Other name: UT575

Primer Pairs:

Primer A: TGGAGGTTGCAATGAGCTG

Primer B: CATGCACACCTAATTGGCG

DNA sequence.

ACGAGGACAGGAGTTCAAGACCAGCCTGGCCAACATGGTGAACCCCGTT
TNTACTAAAANTACAAAANTTGGTCGGGAGGCTGGGGCAGGNGACATGC

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TTGACCCCAAGGAGGTGGAGGTTGCAATGAGCTGAGATTGCACCACTGCA
CTNCAGCNTGG.....AAGAAAGAGAAAGGANAGNNAGGNAGNNANNAAC
TACATNTGAAGTCAACACTAGTATTGGTGGGAGAGGAATTTTATGCTGCA
TTCCCNACAACCACTAGATACGCCAATTAGGTGTGCATGGTCCATGCTA
T

GenBank ID: L26588

3. D18S817.

Other name: UT6365

Primer Pairs:

Primer A: GCAAAGCAGAAGTGAGCATG

Primer B: TAGGACTACAGGCGTGTGC

DNA Sequence:

CATATGGGTCCACAAGCAACCTCAGTCCTTGTCTCTTCAGAAGAAAGAAT
TCTACTGAGGGNCATAAGGCAGAAGGAGAGACCTAGGCAAGTTGCAAAG
CAGAAGTGAGCATGTATTAAGAAAGCTTTAGAACAGTAAGGAAAGGAAGAA
AAGAAAAGAAGGAAAGTTCAACTTGAAGAGGGCCAAGCCGGCAACTTG
GCAGAAGGATTGCTTGAGCCCAGGAGTTAAGACCAGTCTGGGCAATATA
GTGAGACTCCATCTCTGCATACATACATACATACATACATACATACA
TACATACATATTGCAGGGTATGATGGCACACGCCTGTAGTCCTAGCTACT
CTGGAGGTTGAGATGGGAGGGTCACTGAGCCTGGGAANTTGAGGCTGC
NNTGAGCCATGATC

GenBank ID: L30552

0016177202441

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Characterisation of YACs.

8 YACs were selected covering the candidate region and flanking the gap. These YACs were further characterised by determining the end-sequences by the Inverse-PCR protocol.

Selected YACs: 961_h_9, 942_c_3, 766_f_12, 731_c_7, 907_e_1, 752_g_8, 717_d_3, 745_d_2

New STSs based on end-sequences (unless indicated otherwise, the STSs were tested on a monochromosomal mapping pannel for identifying chimaerism of the YAC; if the STS revealed a hit not on chromosome 18q - chimaeric YAC- then it is indicated in the text below):

1. SV32L.

Derived from YAC 745_d_2 left arm end-sequence.

Primer A: GTTATTACAATGTCACCCTCATT

Primer B: ACATCTGTAAGAGCTTCACAAACA

DNA-sequence:

ATTCCTTNGTTATTACAATGTCACCCTCATTAAAAAGTGGAAAGATAAAG
AGGAAGCAATCTATTTTTTCTTTTTTCTGATAGCACTGTTTGGAAG
CTCTTACAGATGTTCTTAAGTAAAATCAACTCCTCCATTTTTTTGTAGCA
ACTACACATATTTATCAATAATAGTTCACAAATACATTTTCAAATT

Amplified sequence length: 107 basepairs (bp)

This STS has no clear hit on the monochromosomal mapping pannel.

2. SV32R.

Derived from YAC 745_d_2 right arm end-sequence.

Primer A: ACGTTTCTCAATTGTTTAGTC

Primer B: TGTCTTGGCATTATTTTTAC

DNA sequence:

AGACAATGGGAGAAATTGCACTGCCCTGAGTCAGAAATCAGATCTGTTG
CCATACAGCTGCCGTTATGTGATCATTTGCAAGTCAACGTTTCTCAATTG
TTTAGTCATTTGTAAGACAAAAAGACTGGTTGGATTTGAGAGAATTTGGA
ATCCTCCTTCAGGTTTAACAAGCAATAAATGATACTCTTCAGTGTAATAAT
AATGCCAAGACATNATTTGACTTTAAATTAAATCCAAACAAGATATC

Amplified sequence length: 127 bp

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This STS has no clear hit on the monochromosomal mapping pannel.

3. SV11L

Derived from YAC 766_f_12 left arm end-sequence.

Primer A: CTATGCTCTGATCTTTGTTACTTT

Primer B: ATTAACGGGAAAGAATGGTAT

DNA sequence:

GTCTTTATTTTCAATAACTATGCTCTGATCTTTGTTACTTTCTCCTTTTAAC
TCAGTTTAAGCTTTATTCTTATTTTCCAGCTGCTGAAGGTATATAGTTAGG
TTGTTTATTGGATACCATTCCTTTCCCGTTAATGTCAGTGGTACTGCTATC
AATGTAGCAGTTA

Amplified sequence length: 118 bp

This STS has a hit with chromosome 18 and must be located between
CHLC.GATA-p6051 and D18S968.

4. SV11R

Derived from YAC 766_f_12 right arm end-sequence.

Primer A: AAGGTATATTATTTGTGTCG

Primer B: AAACCTTTCTTAACCTCATA

DNA sequence:

ATAAGGTATATTATTTGTGTCGTGAGTTAAGAAATCATTAACTATTTT
CAGAATGACAAATGTCATTATATGTTGTAAAAAAGATAAATACGTGAAAT
ATGAGGTTAAGAAAAGTTTA

Amplified sequence length: 119 bp.

This STS has a hit with chromosome 18 and must be located between
D18S876 and GCT3G01.

5. SV34L

Derived from YAC 717_d_3 left arm end-sequence.

Primer A: TCTACACATATGGGAAAGCAGGAA

Primer B: GCTGGTGGTTTTGGAGGTAGG

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DNA sequence:

ACATAAAATGTCGCTCAAAAACAATTATGTGTGTCTACACATATGGGAAA
GCAGGAAACAAATTTGTTTACAACATACATTACTTTTGTITTTTAGGCAAG
ATAAAATNTCTACCTCCAAAACCACCAGCACNGTCCGCAATAACTATAC
ATC

Amplified sequence length: 98 bp

This STS has a hit with chromosome 18.

6. SV34R.

Derived from YAC 717_d_3 right arm end-sequence.

Primer A: ATAAGAGACCAGAATGTGATA

Primer B: TCTTTGGAGGAGGGTAGTC

DNA-sequence:

AATATCATTCTTCACCCACGTTATACATAAGAGACCAGAATGTGATATTGT
CATCTCACATGGAAAAATCTGCTGTGATCAGTTCCTGAAGCTTGCTGTGA
TCCTCCCTTAGGAAAGTAGAAAAATCTTTTGAACACTTTATTCTACAAT
CAATGAAAATTAGGTGAAGCTACAGAAGCCAGAAATTACTCTAAGATTAG
ACAATTATTTAAGANGACCAATTGTCTTTGGTCTTCTTCTGAAGGGTCTG
ACTACCCTCCTCCAAAGAATTCACCTGGCCGTCGTTTTACAACGTCNTGA

Amplified sequence length: 244 bp

This STS has a hit with chromosome 1, therefore YAC 717_d_3 is chimaeric

7. SV25L.

Derived from YAC 731_c_7 left arm end-sequence.

Primer A: AAATCTCTTAAGCTCATGCTAGTG

Primer B: CCTGCCTACCAGCCTGTC

DNA sequence:

AGTGGAGAGATAGAAAGAGAGGAAGATTTTTTTTTTAAATCTCTTAAGCT
CATGCTAGTGTAGGTGCTGGCAGGTCTGAACACTCTGTAGGACAGGCTG
GTAGGCAGGAA

Amplified sequence length: 72 bp

This STS has no clear hits on the monochromosomal mapping panel.

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8. SV25R.

Derived from YAC 731_c_7 right arm end-sequence.

Primer A: TGGGGTGCGCTGTGTTGT

Primer B: GAGATTTTCATGCATTCCTGTAAGA

DNA-sequence:

GGAGGGTGTTNTCACANAAGTCTGGGGTGCGCTGTGTTGTTTCATTGTAA
AAACCCTTTGGANCATCTGGGAATGTGCTGCCCCACATGTCCAGGTAAC
GTTCTCAGGAAGGGGAGGCTGGAAATCTCTGTGTGTTCTTACAGGAATG
CATGAAATCTCCCANCCCCTCTTGTTGGAAATTTCCCTCACTTT

Amplified sequence length: 136 bp

This STS has a hit with chromosome 7; therefore YAC 731_c_7 is chimaeric

9. SV31L.

Derived from YAC 752_g_8 left arm end-sequence.

Primer A: GAGGCACAGCTTACCAGTTCA

Primer B: ATTCATTTTCTCATTTTATCC

DNA-sequence:

CTTCTCNATGANTGGACAAATGTCATTGGGTCAGCATGAGGCACAGCTT
ACCAGTTTCAGATTCCAGTAGCTGAGGAACAAATCTTAACCCAAAAATAA
GTAATTGCGTCACTTTGGAGGAATTATTTGACCTTTTCATAACTTTGACAT
CACAACAATGAGGGTGAAGTTAGTAAATAAATGATTATTATGAGGATAA
AATGAGAAAAATGAATTNAGTGCTTAAGACAATGCTTGGTAACTAGTTAAN
CCG

Amplified sequence length: 178 bp

This STS has a hit with chromosome 18 and must be located between
D18S876 and GCT3G01.

10. SV31R.

Derived from YAC 752_g_8 right arm end-sequence.

Primer A: CAAGATTATGCCTCAACT

Primer B: TAAGCTCATAATCTCTGGA

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DNA sequence:

AAACTTTAACCAATTTAACTCCCTAACAGTTCTATAAAATAAGCAAGATT
ATGCCTCAACTTTATGGATAAAGAAATGGAGGCATTAAGAGATAACTAAC
TTGCCCAAGGCCACACAAGTGAAGTGAAGTAAGAATTGCAAAGCCAATGAG
TCTGGCTCCAGAGATTATGAGCTTAATCACCACACTGTGCCACCTCCTGT
GTTTCCTGG

Amplified sequence length: 131 bp

This STS has no clear hits on the monochromosomal mapping pannel and
gives no information concerning the chimaerity of the YAC.

11. SV10L

Derived from YAC 942_c_3 left arm end-sequence.

Primer A: TCACTTGGTTGGTTAACATTACT
Primer B: TAGAAAAACAGTTGCATTTGATAT

DNA-sequence:

GGTNTTTCACCTGGTTGGTTAACATTACTCTAAGTTTTTTATTGTTTTTA
TGCTATTGCTAATGGGATTGCTTTCTTAATTTATTTTTCCAATAGCTTGT
TGTTAGTTTATATCAAAATGCAACTGTTTTCTATGCAAATTATGTTTCCT

Amplified sequence length: 130 bp

This STS has a hit with chromosome 18 and must be located between
CHLC.GATA-p6051 and D18S968

12. SV10R

Derived from YAC 942_c_3 right arm end-sequence.

Primer A: AACCCAAGGGAGCACAACCTG
Primer B: GGCAATAGGCTTTCCAACAT

DNA sequence:

TTGGTGGTGGCCTAGGTTTGGCAATTATAAATAAAGCTGCTACAAACATT
CATGTGCAGGTCTCCGTGTGGACATAATTTCCAGTTCATTTGGGTAAAA
CCCAAGGGAGCACAACCTGTTGGATCCTATNATAAAAATATNTCTCGTTTC
ATTTAAAAAACCTGGGAAACTATCTNCCCACAGTGGCTGTCCCTTTTGT
ATCCCCACCAACAATGTTGGAAAGCCTATTGCCANCAT

Amplified sequence length: 135 bp

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This STS has a hit with chromosome 18 and must be located between D18S876 and GCT3G01

13. SV6L

Derived from YAC 961_h_9 left arm end-sequence.

No primer was made, because this sequence is identical to a known STR marker D18S42, which is indeed mapped to this region.

Primer A:

Primer B:

DNA sequence:

CATGNCTCACAGTGTCTGAGGCTGCTCTGGACATGCAATCTTGCATGC
TTTTGTCATGACAGGTCTTAAANAGTTTATCAGCTTNCCTCAAATAGCTGAA
TGACANAACACTGGATTTTTGTTCAAATANCCTATCAACTTGGCNCCTGT
GTTGCGGTTGTCACCTTGGTAACAAAATAAGTC

Amplified sequence length:

SV6L recognises D18S42 which must be therefore located between WI-7336 and WI-8145

14. SV6R

Derived from YAC 961_h_9 right arm end-sequence.

Primer A: TTGTGGAATGGCTAAGT

Primer B: GAAAGTATCAAGGCAGTG

DNA sequence:

TAATTGACAAATAAAAATTGTATATTTTNCATATTTAACATGTTATGCTAAC
ATATATATGGATTGTGGAATGGCTAAGTCAGAAATCTTTTACATTCATAT
TTCCATATTATTACTTTNNGCTTTAAAAAATATGTAAATGANAATACTTAT
TTTTTTCAGTGTCACCTTGCCCTTGATACTTTTACATTTNNGTTACATATTATT
CCCTTNCATCTAACAAATATATATTGAGTTTCTATAATGTGTCTGACACTG
A

Amplified sequence length: 122 bp

SV6R amplifies a segment on chromosome 18. This segment must be located between WI-2620 and WI-4211

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15. SV26L.

Derived from YAC 907_e_1 left arm end-sequence.

Primer A: TATTTGGTTTGTGCTGAGGT

Primer B: CAAGAAGGATGGATACAAACAAG

DNA sequence:

TGGTCACTGGTGCCTTATTTGGTTTGTGCTGAGGTCATATTCCTGTG
GCCTTCATGCTTGATTGTTGGAGTCTAGCCATGTAAAANTCTGTTGGAG
TCTAGGCATTTAAAAATAGGTATTTATTGTAATCTTTGCCATTTGCTTGT
TTGTATCCATCCTTCTTGGGAAGGCTTTACAGGCATTCAAAAGG

Amplified sequence length: 154 bp

This STS has a hit with chromosome 13; therefore YAC 907_e_1 is chimaeric.

16. SV26R.

Derived from YAC 907_e_1 right arm end-sequence.

Primer A: CGCTATGCATGGATTTA

Primer B: GCTGAATTTAGGATGTAA

DNA sequence:

CGCTATGCATGGATTTAACTGAGTGTAGTGCACCTCACTATGTTGCAGTC
TCTTATTCTAGGTTCTAATATTTACATCCTAAATTCAGCT

Amplified sequence length: 90 bp

no clear hits on monochromosomal mapping panel: no information concerning chaemerity at this side of the YAC

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Testing of 3 end-sequences flanking the gap in additional YACs: STS-markers WI-4211, D18S876 and GCT3G01 are also shown in order to identify YACs on opposite sides of the gap more clearly in table 3 below.

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YACs	STSs					
	WI-4211	D18S876	SV31L	SV11R	SV10R	GCT3G01
940_b_1	+	+	+	-	-	-
766_f_12	+	+	+	+	-	-
846_a_5	+	-?	+	+	-	-
752_g_8	+	+	+	+	-	-
745_d_2	+	+	+	+	-	-
961_c_1	+	+	-	-	-	-
942_c_3	+	+	+	+	+	-
717_d_3	-	-	+	+	-?	+
972_e_11	-	-	-	-	-	+
940_h_10	-	-	-	-	+	+
821_e_7	-	-	-	-	+	+
731_c_7	-	-	-	-	-	+
889_c_4	-	-	-	-	+	+
907_e_1	-	-	-	+	+	+

20

- +: positive hit / -: no hit / ?: 2 instances were observed in which a positive hit was expected (on the assumed order of the markers) but not observed. The reasons for this are not clear.

25

YAC 745_d_2 was excluded from further analysis since there was no clear hit with chromosome 18. Of the remaining 7 from a monochromosomal mapping panel it was determined that 3 were chimeric and 4 non-chimeric as shown in Table 4 below.

30

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TABLE 4

	YAC	chimaeric	chromosome
5	961_h_9 (6)	no	
	942_c_3 (10)	no	
	766_f_12 (11)	no	
	731_c_7 (25)	yes	chromosome 7
	907_e_1 (26)	yes	chromosome 13
10	752_g_8 (31)	no	
	717_d_3 (34)	yes	chromosome 1

15 For the non-chimeric YACs the STS based on the end-sequence flanking the gap (10R, 11R, 31L) was tested on 14 YACs flanking the gap. Overlaps between YACs on opposite sides of the gap were demonstrated: e.g. the "11R" end-sequence (766.f.12) detects YAC 766.f.12 and YAC 907.e.1.

20 YACs were then selected comprising the minimum tiling path:

TABLE 5

25	YAC	size	chimaerity
	961_h_9	1180 kb	not chimaeric
	766_f_12	1620 kb	not chimaeric
	907_e_1	1690 kb	chimaeric (chr. 13)

30 These three YACs are stable as determined by PFGE and their sizes roughly correspond to the published sizes. These YACs were transferred to other host-

35 yeast strains for restriction mapping.

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Experimental 2Construction of fragmentation vector:

5 A 4.5kb ECORI/SalI fragment of pBLC8.1 (Lewis et
al, 1992) carrying a lysine-2 and a telomere sequence
was directionally cloned into GEM3zf(-) digested with
ECORI/SalI. Subsequently, an End Rescue Site was
ligated into the ECORI site. Hereto, two
10 oligonucleotides (strand 1: 5'-TTCGGATCCGGTACCATCGAT-
3' AND STRAND 2: 3'-GCCTAGGCCATGGTAGCTATT-5') were
ligated into a partial (dATP) filled ECORI site,
generating the vector pDF1. Triplet repeat containing
fragmentation vectors were constructed by cloning of a
15 21bp and a 30bp CAG/CTG adapter into the Klenow-filled
PstI site of pDF1. Trasformation and selection
resulted in a (CAG)₇ and a (CTG)₁₀ fragmentation vector
with the orientation of the repeat sequence 5' to 3'
relative to the telomere.

20

Yeast transformation:

Linearised (digested with SalI) vector was used
to transform YAC clones 961.h.9, 766.f.12 or 907.e.1
25 using the LiAc method. After transformation the YAC
clones were plated onto SDLys⁻ plates to select for
the presence of the fragmentatio vector. After 2-3
days colonies were replica plated onto SDLys⁻-Trp⁻-Ura⁻
and SDLys⁻-Trp⁻-Ura⁺ plates. Colonies growing on the
30 SDLys⁻-Trp⁻-Ura⁺ plates but not on the SDLys⁻-Trp⁻-Ura⁻
plates contained the fragmented YACs.

Analysis of fragmented YACs:

35

Yeast DNA isolated from clones with the correct

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phenotype was analysed by Pulsed Field Electrophoresis (PFGE), followed by blotting and hybridisation with the Lys-2 gene and the sizes of the fragmented YACs were estimated by comparison with DNA standards of known length.

End Rescue:

Fragmented YACs characterised by a size common to other fragmented YACs, indicative of the presence of a major CAG or CTG triplet repeat, were digested with one of the enzymes from the End Rescue site, ligated and used to transform E. Coli. After growth of the transformed bacteria the plasmid DNA was isolated and the ends of the fragmented YACs, corresponding to one of the sequences flanking the isolated trinucleotide repeats, were sequenced.

Sequencing revealed that fragmented YACs of an equal length were all fragmented at the same site. A BLAST Search of the GenBank database was performed with the identified sequences to identify homology with known sequences. The complete sequence spanning the CAG or CTG repeats of the fragmented YACs was obtained by Cosmid Sequencing, employing sequence specific primers and splice primers, as previously described (Fuentes et al. 1992 Hum.Genet. 101: 346-350) or by using the "genome walker" kit (Clontech Laboratories, Palo Alto, USA) and described in Siebert et al. Nucleic Acid Res (1995) 23(6): 1087-1088 and Siebert et al. (1995) CLONTECHniques X(II): 1-3.

Results:

A YAC 961.h.9 clone was transformed with the (CAG)₇ or (CTG)₁₀ fragmentation vector. The CTG vector

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did not reveal the presence of any CTG repeat.

Analysis of twelve (CAG)₇ fragmented YACs showed that five of these had the same size of approximately 100kb. End Rescue was performed with ECORI and sequencing of three of these fragments revealed that they all shared the terminal sequence shown in italics in Figure 15a. A BLAST search of the Genbank database with this sequence indicated the presence of a sequence homology with the CAP2 gene (GenbBank accession number: L40377). The sequence spanning the CAG repeat shown in Figure 15a was obtained by both cosmid sequencing and genome walker sequencing. The sequence was mapped between markers D18S68 and WI-3170 by STS content mapping.

A YAC 766-f-12 was fragmented using the (CAG)₇ or (CTG)₁₀ fragmentation vector. Again the (CTG)₁₀ vector did not reveal the presence of any CTG repeat. Analysis of twenty (CAG)₇ fragmented YACs showed the presence of two groups of fragments with the same size: five of approximately 650kb and two of approximately 50kb.

End Rescue was performed using ECORI on four of the fragmented YACs of 650kb. Sequencing confirmed that they all shared identical 3' terminals, characterised by the sequence shown in italics in Figure 16a. A Blast Search showed homology of this sequence with the Alu repeat sequence family. The sequence spanning the CAG repeat shown in Figure 16a was obtained by cosmid sequencing. The sequence was mapped between markers WI-2620 and WI-4211 by STS content mapping on the YAC contig map.

End Rescue was also performed on the two fragments of 50kb. Sequencing revealed the sequence shown in italics in figure 17a. A Blast Search revealed no

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sequence homology with any known sequence. Cosmid sequencing allowed to identify the complete sequence spanning the CAG repeats, shown in figure 17a. The sequence was mapped between markers D18S968 and
5 D18S875 by STS content mapping on the YAC contig map.

A YAC 907-e-1 clone was transformed with the (CAG)₇ or (CTG)₁₀ fragmentation vector. The (CAG)₇ vector did not reveal the presence of any CAG repeat.
10 Analysis of twenty-six (CTG)₁₀ fragmented YACs revealed that twenty-one of them had the same size of approximately 900kb. End Rescue was performed with KpnI on three fragmented YACS of this size. Sequencing revealed the nucleotide sequence shown in italics in
15 Figure 18a. A Blast Search indicated the presence of an homology of this sequence with the GCT3G0I marker (GenBank accession number: G09484). The sequence spanning the CTG repeat was obtained from the GenBank Database. The sequence was mapped between markers 10R
20 and WI-528.

25

30

35

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CLAIMS:

1. Use of an 8.9 cM region of human chromosome 18q disposed between polymorphic markers D18S68 and D18S979 or a fragment thereof for identifying at least one human gene, including mutated or polymorphic variants thereof, which is associated with a mood disorder or related disorder.
2. Use of a YAC clone comprising a portion of human chromosome 18q disposed between polymorphic markers D18S60 and D18S61 for identifying at least one human gene, including mutated or polymorphic variants thereof, which is associated with a mood disorder or related disorder.
3. The use as claimed in claim 2 wherein said portion comprises the region of chromosome 18q between polymorphic markers D18S68 and D18S979 or a fragment of said region.
4. The use as claimed in claim 2 or 3 wherein said YAC clone is 961.h.9, 942.c.3, 766.f.12, 731.c.7, 907.e.1, 752-g-8 or 717.d.3.
5. The use as claimed in claim 4 wherein said YAC clone is 961.h.9, 766.f.12 or 907.e.1.
6. The use as claimed in any preceding claim wherein said mood disorder or related disorder is selected from the Diagnostic and Statistical Manual of Mental Disorders, version 4 (DSM-IV) taxonomy and includes mood disorders (296.XX, 300.4, 311, 301, 13, 295.70), schizophrenia and related disorders (295, 297.1, 298.9, 297.3, 298.9), anxiety disorders

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(300.XX, 309.81, 308.3), adjustment disorders (309, XX) and personality disorders (codes 301. XX).

7. A method of identifying at least one human
5 gene, including mutated or polymorphic variants
thereof, which is associated with a mood disorder or
related disorder which comprises detecting nucleotide
triplet repeats in a region of human chromosome 18q
disposed between polymorphic markers D18S68 and
10 D18S979.

8. A method of identifying at least one human
gene, including mutated or polymorphic variants
thereof, which is associated with a mood disorder or
15 related disorder which comprises fragmentation of a
YAC clone as defined in any one of claims 2 to 4 and
detection of nucleotide triplet repeats.

9. A method as claimed in claim 7 or 8 wherein
20 said repeated triplet is CAG or CTG.

10. A method as claimed in claim 9 wherein said
repeated triplet is detected by means of a probe
comprising at least 5 CTG and/or CAG repeats.
25

11. A method of identifying at least one human
gene including mutated or polymorphic variants
thereof, which is associated with a mood disorder or
related disorder wherein said gene is present in the
30 DNA comprised in the YAC clones as defined in any one
of claims 2 to 5, which method comprises the step of
detecting an expression product of said gene with an
antibody capable of recognising a protein with an
amino acid sequence comprising a string of at least 8
35 continuous glutamine residues.

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12. A method as claimed in claim 11 wherein said DNA forms part of a human cDNA expression library.

13. A method as claimed in claim 11 or claim 12
5 wherein said antibody is mAB 1C2.

14. A method of preparing a contig map of YAC clones of the region of human chromosome 18q between polymorphic markers D18S60 and D18S61 which comprises
10 the steps of:

(a) subcloning the YAC clones according to any one of claims 2 to 5 into exon trap vectors;

15 (b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other known sequence tagged sequence from the YAC contig described herein, or part thereof consisting of not less than 14
20 contiguous bases or the complement thereof, to detect overlaps among the cosmid vectors, and

(c) constructing a cosmid contig map of a YAC clone of said region.

25 15. A method of identifying at least one human gene or any mutated or polymorphic variant thereof which is associated with a mood disorder or related disorder which comprises the steps of:

30 (a) transfecting mammalian cells with DNA sequences cloned into an exon trap vector as prepared in claim 14;

35 (b) culturing said mammalian cells in an appropriate medium;

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(c) isolating RNA transcripts expressed from an SV40 promoter;

(d) preparing cDNA from said RNA
5 transcripts;

(e) identifying splicing events involving exons of the DNA subcloned into said exon trap vector in accordance with claim 14 to elucidate positions of
10 coding regions in said subcloned DNA;

(f) detecting differences between said coding regions and equivalent regions in the DNA of an individual afflicted with said mood disorder or
15 related disorder; and

(g) identifying said gene or mutated or polymorphic variants thereof which is associated with said mood disorder or related disorder.
20

16. A method of identifying at least one human gene or mutated or polymorphic variants thereof which is associated with a mood disorder or related disorder which comprises the steps of:
25

(a) subcloning the YAC clones according to any one of claims 2 to 5 into a cosmid, BAC, PAC or other vector;

(b) using the nucleotide sequences shown in any one of Figures 1 to 11 or any other known sequence tagged sequence from the YAC contig described herein, or part thereof consisting of not less than 14 contiguous bases or the complement thereof, to detect
35 overlaps amongst the subclones and construct a map

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thereof;

(c) identifying the position of genes within
the subcloned DNA by one or more of CpG island
5 identification, zoo-blotting, hybridization of said
subcloned DNA to a cDNA library or a Northern blot of
mRNA from a panel of culture cell lines;

(d) detecting differences between said genes
10 and equivalent regions of the DNA of an individual
afflicted with a mood disorder or related disorder;
and

(e) identifying said gene which, if
15 defective, is associated with said mood disorder or
related disorder.

17. An isolated human gene, including mutated or
polymorphic variants thereof, which is associated with
20 a mood disorder or related disorder which is
obtainable by the method according to any of claims 7
to 13, 15 or 16.

18. A human protein which, if defective, is
25 associated with a mood disorder or related disorder
which is the expression product of the gene according
to claim 17.

19. A cDNA encoding the protein of claim 18 which
30 is obtainable by the method of any one of claims 7 to
13 , 15 or 16.

20. Use of a probe of at least 14 contiguous
nucleotides of the cDNA of claim 19 or the complement
35 thereof in a method for detection in a patient of a

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pathological mutation or genetic variation associated with a mood disorder or related disorder which method comprises hybridizing said probe with a sample from said patient and from a control individual.

5

21. A nucleic acid molecule which comprises a sequence of nucleotides as shown in any one of Figures 15a, 16a, 17a or 18a.

10

22. A nucleic acid molecule which comprises a sequence of nucleotides which differ from a sequence of nucleotides as shown in any one of Figures 15a, 16a, 17a or 18a only in the extent of trinucleotide repeats.

15

23. A protein encoded by a nucleic acid molecule as claimed in claim 21.

20

24. A protein encoded by a nucleic acid molecule as claimed in claim 22.

25

25. A method of determining the susceptibility of an individual to a mood disorder or related disorder which method comprises analysing a sample of DNA from that individual for the presence of a DNA polymorphism associated with a mood disorder or related disorder in a region of chromosome 18q disposed between polymorphic markers D18S68 and D18S979.

30

26. A method as in claims 25 wherein said DNA polymorphism is a trinucleotide repeat expansion.

35

27. A method as in claim 26 wherein said trinucleotide repeat expansion is comprised in a

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sequence of nucleotides that differ from the sequence of nucleotides shown in any one of Figures 15a, 16a, 17a or 18a only in said trinucleotide repeat expansion.

5

28. A method as in claim 26 or 27 which comprises the steps of:

a) obtaining a DNA sample from said individual;

b) providing primers suitable for the amplification of a nucleotide sequence comprised in the sequence shown in any one of Figures 15a, 16a, 17a or 18a said primers flanking the trinucleotide repeats comprised in said sequence;

c) applying said primers to the said DNA sample and carrying out an amplification reaction;

d) carrying out the same amplification reaction on a DNA sample from a control individual; and

e) comparing the results of the amplification reaction for the said individual and for the said control individual;

wherein the presence of an amplified fragment from said individual which is bigger in size from that of said control individual is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

29. A method as in claim 28 wherein said

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nucleotide sequence to be amplified is comprised in the sequence shown in Figure 15a and said primers have the sequences shown in Figure 15b.

5 30. A method as in claim 28 wherein said nucleotide sequence to be amplified is comprised in the sequence shown in Figure 16a and said primers have the sequences shown in Figure 16b.

10 31. A method as in claim 28 wherein said nucleotide sequence to be amplified is comprised in the sequence shown in Figure 17a and said primers have the sequences shown in Figure 17b.

15 32. A method as in claim 28 wherein said nucleotide sequence to be amplified is comprised in the sequence shown in Figure 18a and said primers have the sequences shown in Figure 18b.

20 33. A method of determining the susceptibility of an individual to a mood disorder or related disorder which method comprises the steps of :

25 a) obtaining a protein sample from said individual; and

 b) detecting the presence of the protein of claim 24;

30 wherein the presence of said protein is an indication of the presence of a susceptibility to a mood disorder or related disorder of said individual.

35 34. A method as in claim 33 wherein said protein is detected with an antibody which is capable of

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recognising a string of at least 8 continuous glutamines.

35. A method as in claim 34 wherein said
5 antibody is mAB 1C2.

36. A nucleic acid as claimed in claim 21 for use
as a medicament in the treatment of a mood disorder or
related disorder.
10

37. A protein as claimed in claim 23 for use as a
medicament in the treatment of a mood disorder or
related disorder.

38. A pharmaceutical composition which comprises
15 a nucleic acid as claimed in claim 21 and a
pharmaceutically acceptable carrier.

39. A pharmaceutical composition which comprises
20 a protein as claimed in claim 23 and a
pharmaceutically acceptable carrier.

40. An expression vector which comprises a
sequence of nucleotides as claimed in claims 21 or 22.
25

41. A reporter plasmid which comprises the
promoter region of a nucleic acid molecule as claimed
in claim 21 or 22 positioned upstream of a reporter
gene which encodes a reporter molecule so that
30 expression of said reporter gene is controlled by said
promoter region.

42. A cell line transfected with the expression
vector of claim 40.
35

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43. An eukaryotic cell or multicellular tissue or organism comprising a transgene encoding a protein as claimed in claims 23 or 24.

5 44. A method for determining if a compound is an enhancer or inhibitor of expression of a gene associated with a mood disorder or related disorder which comprises the steps of:

10 a) contacting a cell as claimed in claim 42 with said compound;

15 b) detecting and/or quantitatively evaluating the presence of any mRNA transcript corresponding to a nucleic acid as claimed in claim 21 or 22; and

20 c) comparing the level of transcription of said nucleic acid with the level of transcription of the same nucleic acid in a cell as claimed in claim 42 not exposed to said compound;

25 45. A method for determining if a compound is an enhancer or inhibitor of expression of a gene associated with a mood disorder or related disorder which comprises the steps of:

30 a) contacting a cell as claimed in claim 42 with said compound;

 b) detecting and/or quantitatively evaluating the expression of a protein as claimed in claims 23 or 24 and

35 c) comparing the level of expression of said

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protein with that of the same protein in a cell not exposed to said compound.

5 46. A method for determining if a compound is an enhancer or inhibitor of expression of a gene associated with a mood disorder or related disorder which comprises the steps of:

10 a) contacting a cell transfected with a reporter plasmid as claimed in claim 41 with said compound;

15 b) detecting or quantitatively evaluating the amount of reporter molecule expressed; and

20 c) comparing said amount with the amount of expression of said reporter molecule in a cell comprising said reporter plasmid and not exposed to said compound.

25 47. A compound identified as an enhancer or an inhibitor of the expression of a gene associated with a mood disorder or related disorder by a method as claimed in claims 44 to 46.

30

09/581500

533 Rec'd PCT/PTO 14 JUN 2000

SEQUENCE LISTING

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ggggtggcaa ccgaagtgtt tctttgggca atggcagcag aattcatgat tgttttcatg 420
trccagcagc agtggcagcg caktgagttg catgattgtt ggctggggct gagtgtctggc 480
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taacctgggc gtgggtggcg gccccagcta ntccggaggc tgaggcagga gaatgggtgtg 180
aacccgggag gcggagcttg cagttagccg agatcccgct actgcaactcc agcctgggca 240
atagagggag actccgtctc aaaaaaaaaa aaaaataaat aataataaaa aaaataacaa 300
taataataact aataattgct tgatatttta caaaagcaaa aggaaaagaa gactaggcaa 360
gaaaaaaaaa acctccttag atggtagaac tcagggtttaa aattaaaact tattctggtg 420
tcagsctagt tgtatatttt gacctcttta aatgctctga actatgatat ggagtaacag 480
cgatgtgctg gctgctgctg ctgctgctga tgggtggtgtt gtttttaatat cgaataaaag 540
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<400> 22

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22

<210> 23

<211> 24

<212> DNA

<213> Homo sapiens

<400> 23

gaactatgat atggagtaac agcg

24

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ABSTRACT

The present invention comprises the use of an 8.9 cM
region of human chromosome 18q disposed between
5 polymorphic markers D18S68 and D18S979 or a fragment
thereof for identifying at least one human gene,
including mutated and polymorphic variants thereof,
which is associated with mood disorders or related
disorders. The invention also provides methods for
10 determining the susceptibility of an individual to
mood disorders or related disorders, comprising
analysing a DNA sample for the presence of a
trinucleotide repeat expansion in the above region.
Polynucleotide sequences useful for detecting the
15 presence of such trinucleotide repeat expansions are
also provided.

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FIG. 1.

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GTCTTTATTTCAATAACTATGCTCTGATCTTTGTTACTTTCTCCTTTTAAC
TCAGTTTAAGCTTTATTTCTTATTTTCCAGCTGCTGAAGGTATATAGTTAGG
TTGTTTATTGGATACCATTCTTTCCCGTTAATGTCAGTGGTTACTGCTATC
AATGTAGCAGTTA

FIG. 2.

ATAAGGTATATTATTTGTGTCGTGAGTTAAGAAATCATTAACTATTTT
CAGAATGACAAATGTCATTATATGTTGTAAAAAGATAAATACGTGAAAT
ATGAGGTTAAGAAAAGTTTA

FIG. 3.

ACATAAAATGTCGCTCAAAAACAATTATGTGTGTCTACACATATGGGAAA
GCAGGAAACAAATTTGTTTACAACATACATTACTTTTGTITTTTAGGCAAG
ATAAAATNTCCTACCTCCAAAACCACCAGCACNGTCCGCAATAACTATAC
ATC

FIG. 4.

AATATCATTCTTCACCCACGTTATACATAAGAGACCAGAATGTGATATTGT
CATCTCACATGGAAAAATCTGCTGTGATCAGTTCCTGAAGCTTGCTGTGA
TCCTCCCTTAGGAAAGTAGAAAAATCTTTTGAACACTTTATTCTACAAT
CAATGAAAATTAGGTGAAGCTACAGAAGCCAGAAATTAATCTAAGATTAG
ACAATTATTTAAGANGACCAATTGTCCTTTGGTCTTCTTCTGAAGGGTCTG
ACTACCCTCCTCCAAAGAATTCACTGGCCGTCGTTTTACAACGTCNTGA

FIG. 5.

GGAGGGTGTTNTCACANAAGTCTGGGGTGCGCTGTGTTGTTTCATTGTAA
AAACCCCTTTGGANCATCTGGGAATGTGCTGCCCCACATGTCCAGGTAAAC
GTTCTCAGGAAGGGGAGGCTGGAAATCTCTGTGTGTTCTTACAGGAATG
CATGAAATCTCCCANCCCTCTTGTTGGAAATTTCCCTCACTTT

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FIG. 6.

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CTTCTCNATGANTGGACAAATGTCATTGGGTCAGCATGAGGCACAGCTT
ACCAGTTCAGATTCCAGTAGCTGAGGAACAAATCTTAAGTCCAAAAATAA
GTAATTGCGTCACTTTGGAGGAATTATTTGACCTTTTCATAACTTTGACAT
CACAACAATGAGGGTGAAGTTAGTAAAATAAATGATTATTATGAGGATAA
AATGAGAAAATGAATTNAGTGCTTAAGACAATGCTTGGTAACTAGTTAAN
CCG

FIG. 7.

GGTNTTTCACCTTGGTTGGTTAACATTACTTCTAAGTTTTTTATTGTTTTTA
TGCTATTGCTAATGGGATTGCTTCTTAATTTATTTTTTCCAATAGCTTGT
TGTTAGTTTATATCAAATGCAACTGTTTTCTATGCAAATTATGTTTCCT

FIG. 8.

TTGGTGGTGCCCTAGGTTTGGCAATTATAAATAAAGCTGCTACAAACATT
CATGTGCAGGTCTCCGTGTGGACATAATTTTCCAGTTCATTTGGGTAAAA
CCCAAGGGAGCACAACTGTTGGATCCTATNATAAAAATATNTCTCGTTTC
ATTTAAAAAACCTGGGAAACTATCTNCCCACAGTGGCTGTCCCTTTTGT
ATCCCCACCAACAATGTTGGAAAGCCTATTGCCANCAT

FIG. 9.

CATGNCTCACAGTGTTCTGAGGCTGCTCTGGACATGCAATCTTGCAATGC
TTTTGTCATGACAGGTCTTAAANAGTTTATCAGCTTNCTCAAATAGCTGAA
TGACANAACACTGGATTTTTGTTCAAATANCCTATCAACTTGGCNTCTGT
GTTGCGGTTGTCACTTGGTAACAAAATAAGTC

FIG. 10.

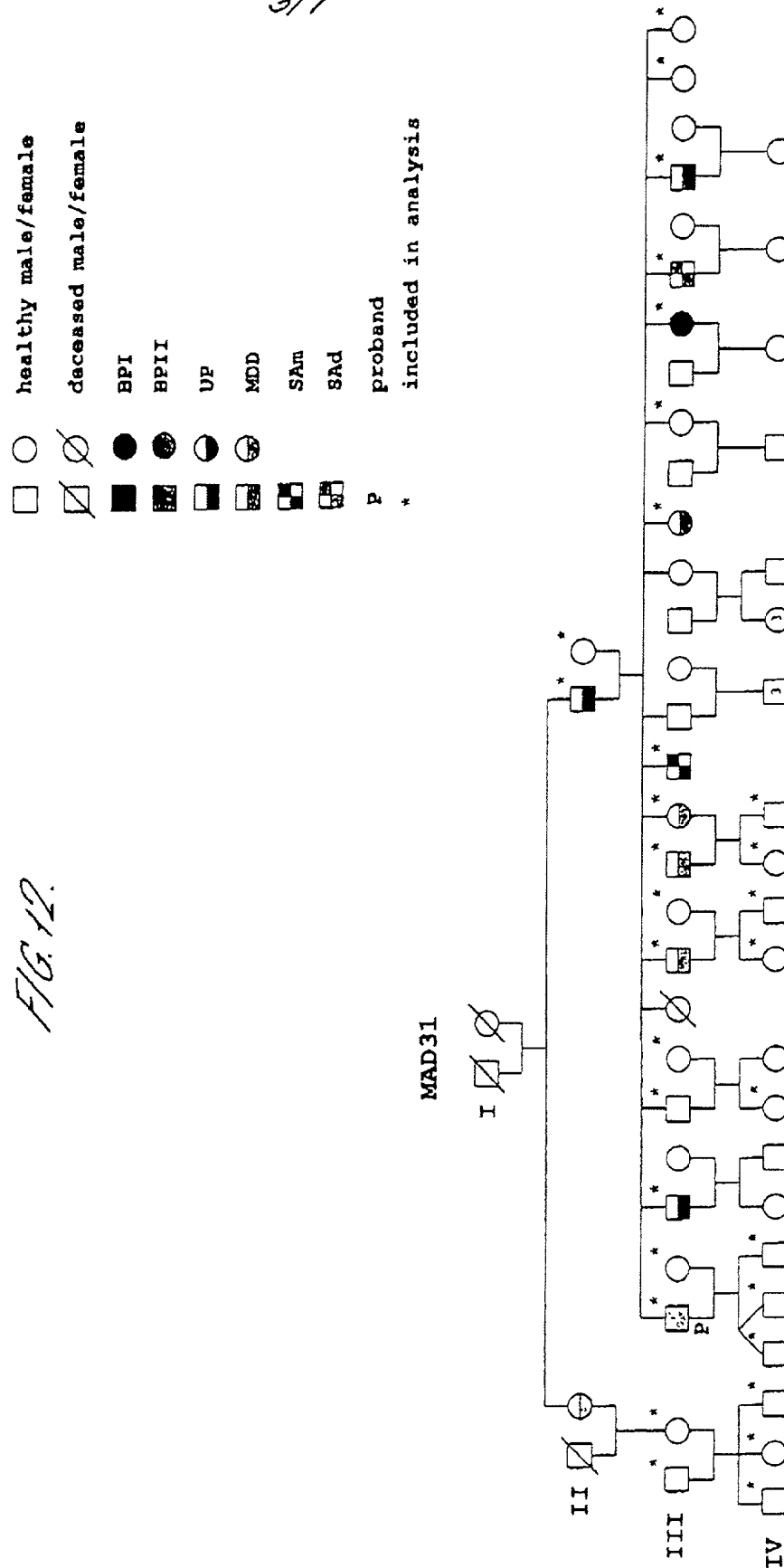
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TTTTTTCAGTGTCACTGCCTTGATACTTTACATTTNNGTTACATATTATTT
CCCTTNCATCTAACAAATATATATTGAGTTTCTATAATGTGTCTGACACTG
A

FIG. 11.

TGGTCACTGGTGCCTTATTTGGTTTGTTTGCTGAGGTCATATTTCTGTG
GCCTTCATGCTTGATTTGTTGGAGTCTAGCCATGTAAAANTCTGTTGGAG
TCTAGGCATTTAAAAAATAGGTATTTATTGTAATCTTTGCCATTTGCTTGT
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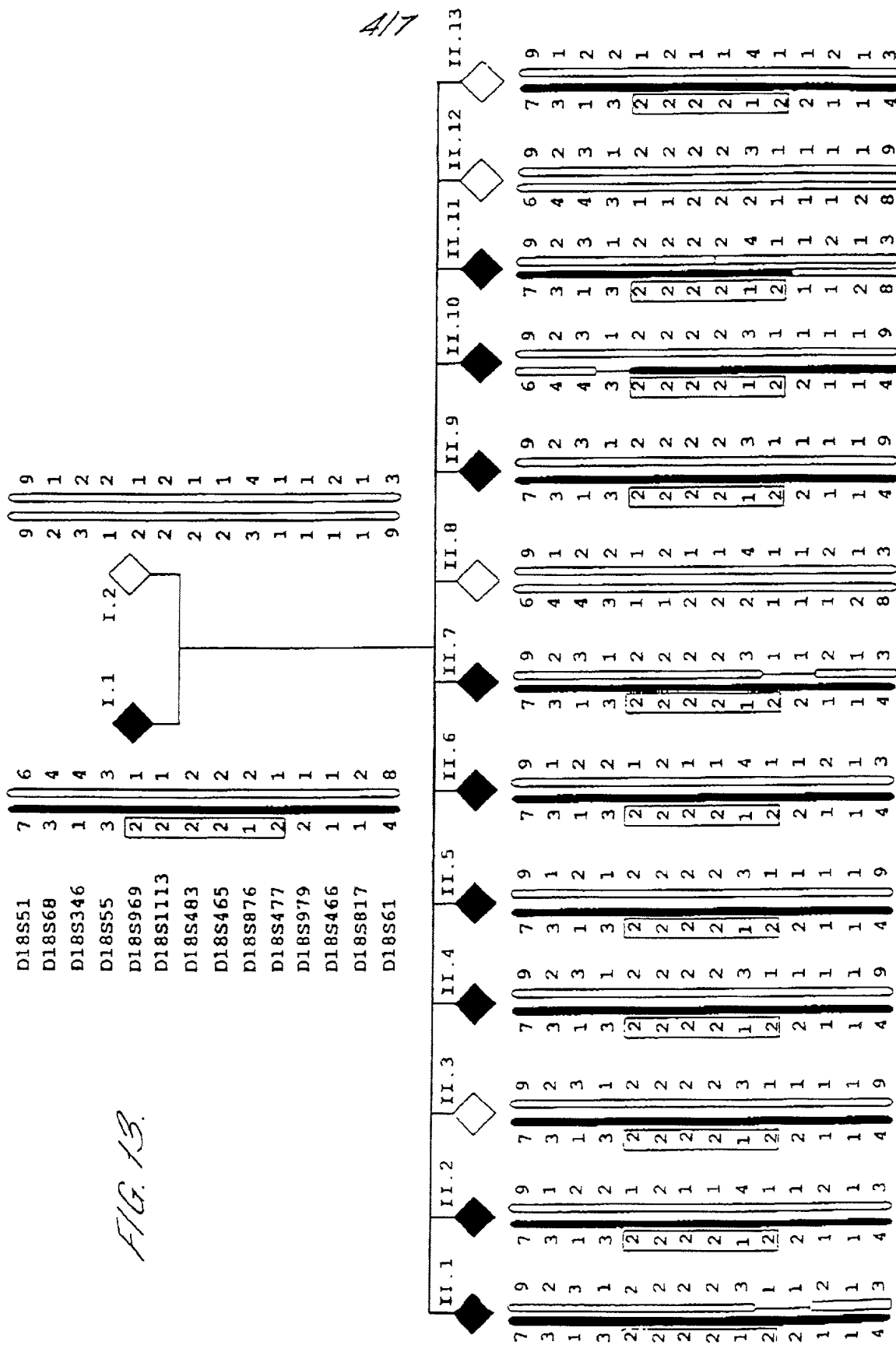
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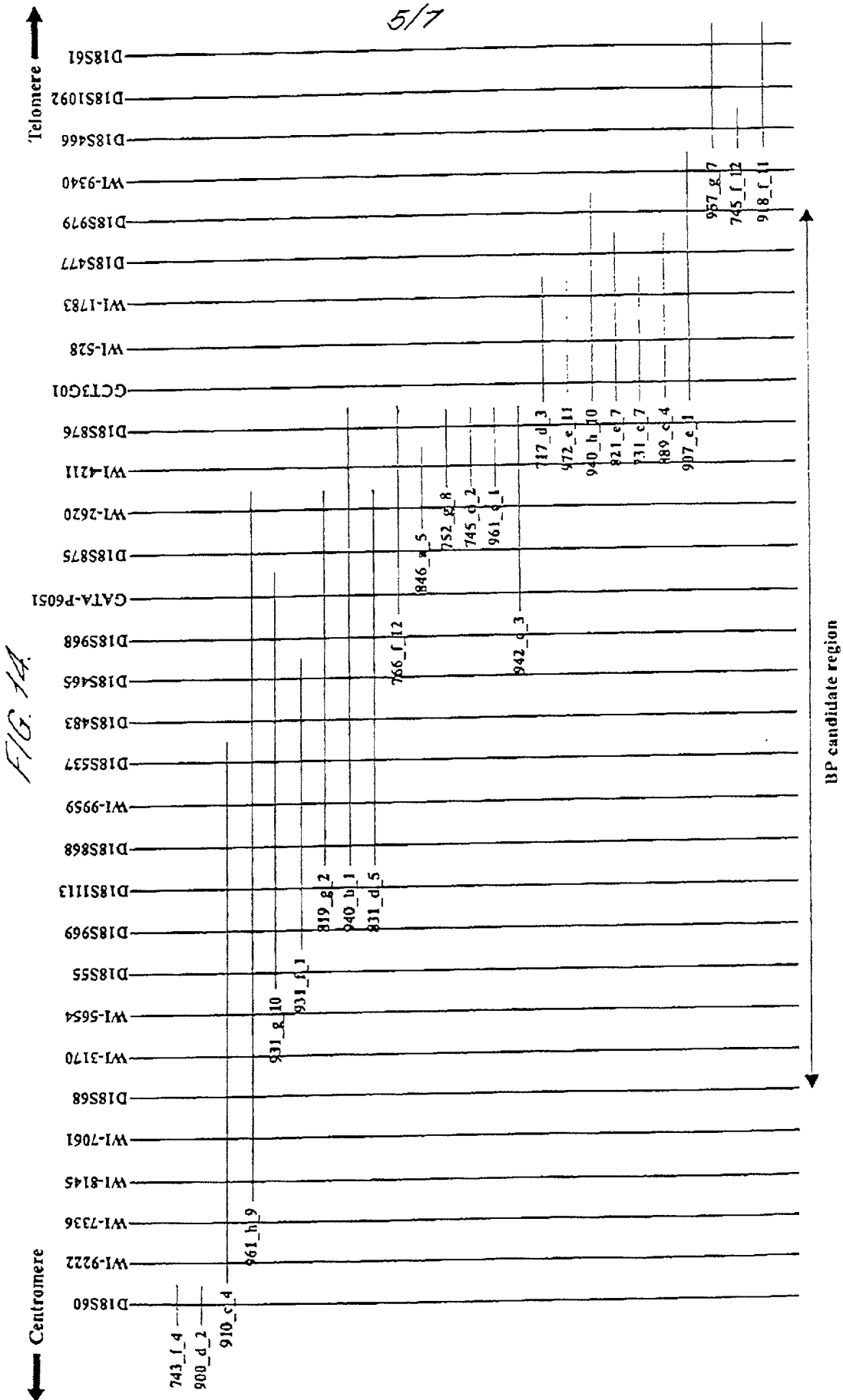
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FIG. 15a.

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GCCAACAAACAAAATGAAATAAGACCTGGGATGTATTTTTGGCCAAGGCAATTAGAAA
 ATGATTAGTATCCTTATCAGGAGCAATTTTCAGAGAATGTTTGGGTGGACGTCTAACTACA
 GTGGAGTCAAACGTGAATCAACGGTGAAAAAAGGACAATAGCCAATGTGTACACTTTTT
 ATAAAAACCACCCTCCAAGGACCAGGCACTGGCCCTCTCTCCGGTGCACACAGACATC
 CACACAGGCCCAAAGAATCAGGGATTGCACAAGCCAGAGCAATCGAACGGTTCTGAGT
 CATCTGCCGGAAGCCTTGCCCTCAATCAAGGCGGACGTGAAGCATCTACAAAGGAGGA
 ATAGTCAAAGCAGCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
 AGGTGGGGGGCCTCTGCCAGGTACCGGGCGGGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
 CCCGCGGAGGCCACCTCTTCCCTGGAGTGCGTGAGAGAGGGGAAGGGAGGAAGG
 CCAGAGCAGGAATCAGAGCGAGGCAAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
 GCGGGAGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
 AGCCGGGGTCCCAAGCCCCACCGCGAGACCCCGCGA

FIG. 15b.

5'-ATCGAACGGTTCTGAGTCATCT
 5'-CGCTCTGATTCTGCTCTG

FIG. 16a.

TTCAGTAGAAGGAAGCACAGCAAATTTGCCCTTTATAGAGATTCAATTCTTGGTGCTTGG
 GCCAAAGAATAAGAATTACATTAAGCAGGCCGGGCACGGTGGCTCACACCTGTAAAAC
 CAGAACTTTGGGAGGCCGAGGCAGGCAGATCATGAGGTCAGGAGATCGAGACCATCC
 TGGACAACATAGTGAAACCCCATCTCTACTAAAAATACAAAAATTAGCCGGGCATGGTG
 GTGCATGCCTGTAATCCCAGCTACTCAGGAGGCGGAGGCAGGAGAATCCCTTGAACCA
 GGGAGTTGGAGTTGCAGTGAGCCGAGATCACGCCACAGCACTCTAGCCTGGCGACA
 GAGTGAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAAAAAAATTACATTAAGCAGCAGC
 AGCAGCAGTGASAGAGGGAAKAATGAAAGAAGAAATTTCTAGAATAAGATTGA
 TCTCCAGCACCATGCCAATCATGGACTGGATACAATTCATGCATATCTTTTGTGA
 GAGAGGTGAGAGATGTGAATCCTTTCTCATT

FIG. 16b.

5'-AGAAGGAAGCACAGCAAATTTG
 5'-GCATGGTGCTGGAGATCAAT

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FIG. 17a.

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TGGGAGTTAAAGCAGACATTTCGGCTTTNGTGTGGCCAGAGTTCTAACATAAGTTCTTTTT
CATCTGGGCAGGCNGATGTTCTTCCATCTTNGAAGNACNGTCTTTTCATTTTTTTAT
TTNGCTTTTGGSKTTTATCTTCTTAGACGTCTTCAGGAGTTKGATTGTAGKGTAAAGGCAG
ATTAGTTGACTGGGCTTTGTTTCTGGAAAATTTAAAGGGGCAAGTCCTGGGCTGCAT
ATTCTTACTCTGGGGGCTTAGTACTGGCCCCCTAAATTTGTTCTCTGGCTCCTCAAGGTT
AGAAATCTGCTGGCTGGAGGGGCTGAGATGTTCTTGGCTGCTGGCCAGAACATTCCTG
CCGGGGGGTGGCAACCGAAGTGTTTCTTTGGGCAATGGCAGCAGAATTCATGATTGTT
TTCATGTRCCAGCAGCAGTGGCAGCGCAKTGAGTTGCATGATTGTTGGCTGGGGC
TGAGTGCTGGCASGCACTGGAGTGTTTGGCTTCCAGTAGAAATTCACAGCAGTAG
TAGTGGTGGCATGGGAAGGAGGGCAGYGGTGGCATGGGGAGGACCCCCC

FIG. 17b.

5'-GGCTGAGATGTTCCCTTGA CTGC

5'-CCTTCCCATGCCACCACTACTA

FIG. 18a.

TGTAATCCCAGCAATTTGGGGAGCCCAAGGCGGGCAGATTCATGAGTTCGGGAAGAT
TCGAGACCNTTCTGGCTAAACACGGGGGAAACCCNTTTTTACTAAAAAATACCAAAA
AATTAACCTGGGCGTGGTGGCGGGCCCCAGCTANTCCGGAGGCTGAGGCAGGAGAAT
GGTGTGAACCCGGGAGGCGGAGCTTGCACTGAGCCGAGATCCCGCTACTGCACTCCA
GCCTGGGCAATAGAGGGAGACTCCGTCTCAAAAAAAAAAAAAAAAAATAATAATAATAAAA
AAAAATAACAATAATAATACTAATAATTGCTTGATATTTACAAAAGCAAAAGGAAAAGAAAG
ACTAGGCAAGAAAAAAAAAAACCTCCTTAGATGGTAGAACTCAGGTTTAAATTAATACTT
ATTCTGGTGTGAGSCTAGTTGTATATTTGACCTCTTTAAATGCTCTGAACATATGATATGG
AGTAACAGCGATGCTGCTGCTGCTGCTGCTGCTGCTGATGGTGGTGGTGTTTTA
ATATCGAATAAAAGTTGTGGAACTAAATTTCAATTTCTGCCAATTAATAAGATT
GCAAAGTTAAACATCT

FIG. 18b.

5'-TTTGCAATCTTAGTTAATTGGC

5'-GAACTATGATATGGAGTAACAGCG

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MOOD DISORDER GENE

the specification of which is attached hereto unless the following is checked:

☒ was filed on June 14, 2000, as Application No. 09/581,500, bearing attorney docket No. B0192/7019, and was amended on June 14, 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or section 365(a) of any PCT International application designating at least one country other than the United States listed below and have also identified below any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign PCT International Application(s) and any priority claims under 35 U.S.C. §§119 and 365(a),(b):

			Priority Claimed	
<u>9726804.9</u> (Number)	<u>Great Britain</u> (Country-if PCT, so indicate)	<u>18 December 1997</u> (DD/MM/YY Filed)	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
<u>PCT/EP98/08543</u> (Number)	<u>PCT</u> (Country-if PCT, so indicate)	<u>17 December 1998</u> (DD/MM/YY Filed)	<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
<u> </u> (Number)	<u> </u> (Country-if PCT, so indicate)	<u> </u> (DD/MM/YY Filed)	<input type="checkbox"/> YES	<input type="checkbox"/> NO



I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

<u>(Application Number)</u>	<u>(filing date)</u>
<u>(Application Number)</u>	<u>(filing date)</u>

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

<u>(Application No.)</u>	<u>(filing date)</u>	<u>(status-patented, pending, abandoned)</u>
<u>(Application No.)</u>	<u>(filing date)</u>	<u>(status-patented, pending, abandoned)</u>

PCT International Applications designating the United States:

<u>(PCT Appl. No.)</u>	<u>(U.S. Ser. No.)</u>	<u>(PCT filing date)</u>	<u>(status-patented,pending,abandoned)</u>
------------------------	------------------------	--------------------------	--

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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